Pica 8: Refining dietary reconstruction through amino acid $\delta^{13}C$ analysis of tendon collagen and hair keratin

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Highlights:

A method for assessing a terrestrial or marine origin for dietary intake is proposed

The new method uses $\delta^{13}C$ values of phenylalanine, valine, and leucine

Tendon collagen is a favourable substitute to bone collagen in dietary reconstructions

Tendon is an ideal tissue for characterising the final year of an individual's life

Abstract

Stable isotope analysis of archaeological human remains is routinely applied to explore dietary habits and mobility patterns. The isotope information pertaining to the period prior to death may help in identifying locals and non-locals, especially when investigating individuals from the same funerary context but believed to have been highly mobile across the landscape.

Based on the variety of the funerary goods in graves and what it is believed their diets comprised, it is thought that both local and non-local individuals were buried at the inland funerary site of Pica 8 (northern Chile, Late Intermediate Period, ~1,050-500 BP); however, uncertainties over the dietary intakes and mobility histories of these individuals still persist. The aim of this study is to refine the dietary characterization of a subset of Pica 8 individuals by increasing the temporal resolution of their dietary reconstructions, specifically throughout the last period of their life, and by identifying the multiple sources of food in their overall diets. This is achieved by analysing the amino acid carbon isotope composition of hair keratin and, for the first time, that of tendon collagen.

This study proposes a new method for identifying the predominant food source (terrestrial or marine) in a mixed diet using phenylalanine, valine and leucine $\delta^{13}C$ values measured in collagenous tissues. Herein, tendon is proven to be an ideal tissue for isotopically characterising the final year of an individual's life. Our results show that individuals previously identified as non-locals, based on long-term food consumption, had in reality abandoned their original dietary habits typical of distant regions many months before death, and hence had presumably relocated to the locality of Pica.

Keywords: Chile; tendon; collagen; amino acid; LC-IRMS; stable isotope; palaeodiet.

1. Introduction

Stable isotope analysis is routinely applied to archaeological human remains for characterising dietary habits and mobility patterns of past populations (Makarewicz and Sealy, 2015). In recent years, attention has focussed on the reconstruction of the life histories of individual identities in past societies (Knapp and van Dommelen, 2008). The reconstruction of an individual's life at different points over their life course is achieved by isotopically analysing multiple tissues (skeletal and non-skeletal) from the same individual, which have differential deposition times and/or turnover rates (Lynnerup, 2007). In particular, the analysis of soft tissues, that have fast remodelling rates, gives information pertaining to the last year/months of life (Lamb, 2015). Moreover, hair retains an unaltered isotope signal locked into the keratins when the tissue was growing (Petzke et al., 2010). This isotope information pertaining to the period prior to death is important when investigating individuals from the same funerary context but believed to have been highly mobile across the landscape, or having had access to long-distance resources. Individuals identified as non-local because of their diet, based on stable isotope compositions averaged over several years (from, for instance, bone collagen) may, in reality, have been consuming locally accessible resources and been part of the local community for a considerable period of time before death.

At the inland funerary site of Pica 8 in northern Chile (Late Intermediate Period, ~1,050-500 BP), uncertainties over dietary intakes and mobility histories of the buried individuals still persist, especially pertaining to the last period of their life. Previous archaeological and biomolecular studies (Núñez, 1984, Petruzzelli et al., 2014, Santana-Sagredo et al., 2015a) have suggested that the individuals buried at Pica may have had different geographical origins and/or a high degree of mobility. Núñez (1984), who first excavated the cemetery, identified the presence of non-local items among the funerary inclusions in the graves (e.g. pottery, textiles, bird feathers, foods). These were thought to have been imported from either the eastern Andes, Altiplano, Azapa valley, or from the coast. Stable isotope analyses of bone and tooth enamel have identified three major dietary related groups of people, consisting of: (1) individuals relying mainly on marine resources, complemented by some maize, (2) individuals relying mainly on maize, complemented by marine resources, and (3) individuals consuming predominantly C_3 terrestrial resources (Santana-Sagredo et al., 2015a).

Based on the variety of grave goods and diets, it can be argued that the people buried at Pica 8 represent a combination of local and non-local individuals who were involved in inter-regional exchange of foodstuffs and exotic objects, and/or sedentary individuals who benefited from having access to a broad range of resources and maintained dietary habits distinctive of their place of origin. Despite the paucity of information surrounding the placement of the burials in the cemetery, there is some evidence (i.e. broad distribution of funerary goods and diets) that the Pica 8 cemetery was divided into ten sectors, A to J (Núñez, 1984, Santana-Sagredo et al., 2015a, Zlatar, 1984), where people with common geographic origins, socio-economic status, and/or cultural traits may have been interred.

In light of new radiocarbon dates of paired human and camelid tissues and estimated ^{14}C offsets, Santana-Sagredo and colleagues (2017) have reconsidered the original dietary interpretation (Santana-Sagredo et al., 2015a) of the Pica individuals, proposing the consumption of C_4 crops fertilised with seabird guano as a major cause for the high $\delta^{15}N$ values (>20‰) measured in bone collagen, rather than the direct consumption of marine resources. Andean archaeological and ethnohistoric records (Covey, 2000, Denevan, 2001, Julien, 1985, Marcus et al., 1999) report that guano was traditionally mined and marine fish were procured and dried by coastal communities and transported to the highlands via llama caravans. Estimating the proportion of guano-fertilised maize and marine fishes in the diet of the Pica individuals is not straightforward since the practice of fertilising maize with guano increases the plant $\delta^{15}N$ values to as much as 20‰, but does not affect $\delta^{13}C$ values (Szpak et al., 2012a, 2012b). As a result, consumption of high-trophic level marine resources (fish and mammals) and guano-fertilised C_4 crops may generate similar ranges of bulk $\delta^{13}C$ and $\delta^{15}N$ values in human tissues (Szpak et al., 2012b). Single amino acid carbon isotope analysis may help in identifying these diverse sources (marine, terrestrial) of food macronutrients (protein, carbohydrate, lipid) by comparing the $\delta^{13}C$ values of essential and non-essential amino acids, given that different metabolic pathways are involved in the processes of assimilation or synthesis of the amino acids making up whole proteins (Newsholme et al., 2011, Reeds, 2000), and that guano is not affecting the carbon stable isotope compositions of plant tissues (Szpak et al., 2012a).

The aim of the present study is to refine the dietary characterization of a subset of Pica 8 individuals by increasing the temporal resolution of palaeodietary reconstructions, specifically throughout the last period of their life, and by identifying the different sources of food (marine and terrestrial) that likely comprised their mixed dietary intakes. This is achieved by analysing the amino acid carbon isotope composition of hair keratin and, for the first time, that of tendon collagen. Tendon has the potential to be an ideal substrate for characterising the period leading to/close to the time of death, considering that (1) collagen is significantly more abundant in tendons (~677+57 nmol/g wet tissue) than in the dermis (~335+64 nmol/g wt), bone (~307+71 nmol/g wt) or skeletal muscle (~59+17 nmol/g wt) (Kjaer et al., 2005), and that (2) the rate of collagen turnover in tendon (Babraj et al., 2005, Miller et al., 2005) is more rapid than in bone (Hedges et al., 2007).

2. The cemetery of Pica 8

Pica 8 is located approximately 80 km inland, at circa 1,300 m of elevation, on the plain of the *Pampa del Tamarugal* (northern Chile, Fig. 1) (Jayne et al., 2016). This funerary site comprises 254 burials (Núñez, 1984) and dates to the Late Intermediate Period (~1,050-500 BP), based on ceramic seriation and radiocarbon dating (Núñez, 1976, Santana-Sagredo et al., 2017, Uribe et al., 2007). The Pica oasis was part of a complex system of communities situated in the Tarapacá region between the Río Camiña in the north and the Río Loa in the south, and covered an altitudinal transect from the coast to the Precordillera (~2,500 masl) (Uribe et al., 2007).

[Figure 1. Map of northern Chile showing the location of the Pica 8 site.]

During this period, the Pica-Tarapacá oases were connected to each other by a complex network of routes and campsites, which allowed long-distance trade of resources and exotic objects between the coast and the highlands, via llama caravans (Briones et al., 2005, Pomeroy, 2013). In this arid region, inter-regional redistribution of surpluses between ecologically different zones was crucial, especially in times of shortage of staple resources (Núñez and Dillehay, 1979, Zori and Brant, 2012). Competition over the control of this trade network likely generated disputes between the local elites. The fact that artefacts related to conflict (found as grave goods) were not associated with violence-related bone injuries, notwithstanding that soft tissue injuries may have occurred, suggests that underlying tension was sublimated in symbolic celebrations of power as a means to reinforce the leadership of certain elites (Pacheco and Retamal, 2017).

3. Materials

The Pica 8 collection is curated at the Department of Anthropology, University of Chile, Santiago de Chile (Lemp et al., 2008), and comprises approximately 150 naturally mummified individuals. Six adult individuals with varying ages, four females and two males, were selected for analysis in order to include bodies deposited in burial sectors that have been suspected to be linked to people with a diversity of diets, geographical origins and cultural identities (Núñez, 1984, Retamal et al., 2012, Santana-Sagredo et al., 2015a). A foot tendon and a bundle of scalp hair were sampled from each individual.

4. Methods

4.1. Tendon collagen stable isotope analysis

Tendon was processed following the Finucane method (2007) originally proposed for soft tissues such as skin and muscle, but increasing the number of washing steps before protein denaturation. In detail, the tendons were cleaned by sonication in Milli-Q water (Merck), immersed in a mixture of chloroform:methanol (2:1 v/v) (Merck), sonicated for ~20 min, and soaked overnight to remove any lipid content. Every 24 hours, the chloroform:methanol (2:1 v/v) was replaced and sonicated (20 min) until all lipids were removed. The samples were then rinsed six times with Milli-Q water (sonicated each time for 20 min). A few drops (~2-3) of 0.5M HCl (Merck) were added to the samples immersed in fresh Milli-Q water to obtain a pH 3 solution. Sealed tubes were placed into a heater block for 48 h at 73°C for gelatinization. The supernatant was filtered with an Ezee-Filter™ (Elkay Laboratory Products), and the residue was discarded. Each filtered sample was then decanted into Pyrex® glass tubes (Corning), frozen (-20 °C), and then freeze-dried (48 h).

4.1.1. Bulk carbon and nitrogen stable isotope analysis

The lyophilised collagen was inserted into tin capsules for the analysis of carbon and nitrogen isotope compositions, which was performed using a Carlo Erba CE1110 CHN-S analyser coupled to a Fisons Isochrom Continuous-flow Isotope Ratio Mass Spectrometer (GV instruments). The δ^{13}C and δ^{15}N values are reported relative to international standards: V-PDB and AIR, respectively. Secondary (reference) materials and in-house standards (MZ1 Maize, HAR4 Sunflower, ATP12) were used to monitor analytical precision of the carbon and nitrogen isotope ratio measurements that were ±0.1‰ (1σ) for both elements.

4.1.2. Single amino acid carbon isotope analysis

Approximately 1 mg of tendon collagen was hydrolysed under vacuum in 1 ml of amino acid-free 6M HCl (Sigma-Aldrich) at 110 °C for 24 h, after Choy et al. (2010). The hydrolysates were dried in a rotary vacuum concentrator and frozen until required for analysis. Prior to analysis, samples were dissolved under sonication in Milli-Q water, with the addition of 10 µl of 1mmol solution of 2-aminoisobutyric acid (Sigma-Aldrich) as the internal standard (I.S.); this was to obtain a sample stock of ~1.8 µg/µl. The sample stock was further diluted in Milli-Q water to deliver 10.8 to 16.2 µg of protein on the LC column (10 µl partial loop injection mode).

LC-IRMS analysis was undertaken at the La Trobe Institute for Molecular Sciences (LIMS, La Trobe University, Melbourne, Australia) using an Accela 600 pump connected to a Delta V Plus Isotope Ratio Mass Spectrometer via a Thermo Scientific LC Isolink (Thermo Scientific). The amino acids were separated using a mixed-mode Primesep A column (2.1x250 mm, 100 Å, 5 µm, SIELC Technologies) following the chromatographic method described in Mora et al. (2017), after Smith et al. (2009). Mobile phases were: Phase A = 35 µl of 1:50 96% H_2SO_4 (Merck) in 1L Milli-Q water, Phase B (1L)= 1 ml 96% H_2SO_4 (Merck) and 2.28 g of ≥98% K_3PO_4 (Sigma-Aldrich) in Milli-Q water, and Phase C (1L)= 3 ml 96% H_2SO_4 (Merck) in Milli-Q water. The LC gradient program was similar to that of Mora et al. (2017), but the flow rate of the analytical run was decreased to 60 - 60 µl/min (Table 1), in order to improve the baseline resolution of the isoleucine, leucine, lysine, histidine and tyrosine peaks. When needed, the flow rate was increased to 80 or 100 µl/min after the tyrosine peak to gain faster sample elution.

[Table 1. Here]

Samples were analysed in duplicate. Baseline separation was achieved for all the amino acids in the tendon collagen, with the exception of methionine (Fig. 2). Histidine and methionine peaks were too small to generate reliable δ^{13}C values. Overall, the amino acid carbon contribution measured by LC-IRMS analysis corresponded to 98.4% of the carbon present in human tendon collagen (Schofield et al., 1971).

[Figure 2. LC-IRMS chromatogram of tendon collagen hydrolysate (from individual SE-T3).]

4.2. Bayesian stable isotope mixing model: FRUITS

The Bayesian mixing model FRUITS - Food Reconstruction Using Isotopic Transferred Signals (Fernandes et al., 2014) has been applied to the collagen stable isotope data produced in the current and previous studies (Santana-Sagredo et al., 2015a) to achieve qualitative and quantitative estimates of the nutritional intake of the Pica individuals. Although other Bayesian stable isotope mixing models have comparable features to those of FRUITS (Phillips et al., 2014), we have used FRUITS as it has been commonly preferred by researchers for reconstructing human palaeodiets, especially from Chilean contexts (e.g. Andrade et al., 2015, King et al., 2018, Mora et al., 2017, Pestle et al., 2016, Pestle et al., 2017) in recent years. Details can be found in Appendix A.

4.3. Hair keratin amino acid carbon isotope analysis

Amino acid $\delta^{13}C$ analysis was performed on 1 cm segments of a single hair from each individual, cut longitudinally along the hair fibre, from the root to the first 10 cm. Hair samples were inserted into hydrolysis tubes, and left to soak in methanol for 12-24 h in order to remove organic residues. Once dried, the hair segment was hydrolysed in 6 M hydrochloric acid at 110°C until the hair fibre was completely dissolved. The amount of 6M HCl used for hydrolysis was increased to ~0.175 μg/μl, compared to Mora et al. (2017). Hydrolysed samples were then dried overnight at 30°C in a rotary vacuum concentrator and frozen until required for analysis. For LC-IRMS injection, samples were dissolved under sonication in Milli-Q water, supplemented by an internal standard (2-aminoisobutyric acid), to obtain keratin hydrolysates with a concentration of approximately 0.8 μg/μl. Samples were analysed in duplicate. The LC-IRMS analysis is detailed in section 4.1.2.

5. Results and Discussion

5.1. Elemental and molecular composition of tissues

5.1.1. Assessment of the preservation of tendon collagen

The calculated C/N atomic ratios (3.2 to 3.4, Table 2) fall within the range of 2.9-3.6, proposed by DeNiro (1985) for well-preserved bone collagen. This range is likely applicable to tendon collagen as the most abundant protein in both tissues is type I collagen (Kannus, 2000, Wang, 2006), and the C/N ratios measured by Ambrose (1990) in bovine tendon were comparable to those measured in bone collagen. The content by weight (%) of carbon (45.8±0.7%) and nitrogen (16.0±0.2%) of the tendons analysed herein is comparable to that measured in bovine tendon collagen (47.6±1.5 %C and 16.0±0.6 %N) by Ambrose (1990). Based on these criteria, the tendon collagens submitted for analysis were well preserved.

[Table 2. here]

5.1.2. Assessment of the molecular preservation of tendon collagen

To assess the molecular preservation of tendon collagen samples, the amino acid Area All [V] values (i.e. sums of the peak areas for the ion currents at *m/z* 44, 45, 46), generated by LC-IRMS, were converted to fractions of the total (%) and compared to (1) those derived from archaeological human bone collagen (n=12, excluding tyrosine) analysed under similar chromatographic conditions, and to (2) the amino acid carbon weights (%) of human tendon collagen estimated from the amino acid residues published by Schofield et al. (1971). The residues were firstly multiplied by the number of carbon atoms per residue to determine the amino acid carbon contribution, and the fraction of the total was calculated to make the residues comparable to the LC-IRMS output. Based on this comparison, it was possible to show that the tendon collagen samples had preserved their amino acid composition. Amino acid profiles (mean±1σ) are shown in Fig. 3.

[Figure 3. Fractions of the total (%) of amino acid peak areas measured in archaeological tendon collagen (this study) and bone collagen (mean±1σ), and of amino acid carbon weights (%) of human tendon collagen derived from Schofield et al. (1971).]

5.1.3. Assessment of the molecular preservation of hair keratin

To assess the molecular preservation of the hair keratins, the amino acid peak areas were converted to fractions of the total (%) and compared to (1) those of modern human hair prepared following the same procedure as for the archaeological hair, but preceded by methanol:chloroform (2:1 v/v) washings to remove any residue of detergents and lipids (O'Connell et al., 2001), and to (2) the amino acid carbon weights (%) of human hair derived from Wolfram (2003) and Robbins and Kelly (1970). Based on this comparison, it was possible to assess that the hair keratin samples had preserved their amino acid composition. Amino acid profiles (mean±1σ) are shown in Fig. 4.

[Figure 4. Fractions of the total (%) of amino acid peak areas measured in archaeological and modern hair (mean±1σ), and of amino acid carbon weights (%) measured in human hair (Robbins and Kelly, 1970, Wolfram, 2003).]

5.2. Bulk stable isotope compositions and FRUITS diet estimates

The δ^{13}C and δ^{15}N isotope values measured in this study from tendon collagen were compared to those measured by Santana-Sagredo et al. (2015a) in bone collagen from the same individuals (Table 2). Due to the differential turnover rates, rib collagen isotope composition represents an average of the dietary intake over the last three to five years of life (Jørkov et al., 2009), while tendon collagen reflects the food consumed during the last few months of life (Babraj et al., 2005). Previous carbon and nitrogen isotope studies (Basha et al., 2016, Finucane, 2007, Iacumin et al., 1998, White and Schwarcz, 1994) on bone and soft tissues (tendon, muscle, skin) from archaeological human remains have reported differences in the isotope values between tissues from the same individual. Multiple and concurrent factors may be responsible, including: (1) changes in diet and/or in physical condition experienced by an individual at different points in his/her life course (Neuberger et al., 2013, Reitsema, 2013, Warinner and Tuross, 2010), (2) different protein (and amino acid) composition of the tissues (e.g. type I collagen vs. actin and myosin) (Eastoe, 1955, Schofield et al., 1971), and (3) diverse fractionation processes that may be connected to the remodelling of the various proteins and/or to their specific metabolism (Ambrose and Norr, 1993, Hare et al., 1991, Tieszen et al., 1983). If it is assumed that different fractionation processes exist for bone and tendon collagen, connected to their specific metabolism and remodelling, these effects are likely to be much smaller than the changes in diet experienced by individuals at different points in their life course, which are differentially recorded in tendon and bone, depending on their turnover rates. Having taken the above into account, the diet-to-collagen offset for tendon collagen is assumed herein to be comparable to that of bone.

Given these premises, the Bayesian mixing model Food Reconstruction Using Isotopic Transferred Signals (FRUITS) (Fernandes et al., 2014) was applied to the tendon collagen isotope data produced in this study and to the bone collagen isotope data published by Santana-Sagredo et al. (2015a). The food groups used in the mixing model include the widest array of foods that were available through trade from the Pacific coast, coastal valleys, mid-altitude valleys and highlands (Aufderheide et al., 1994, Burress et al., 2013, DeNiro and Hastorf, 1985, DeNiro, 1988, Falabella et al., 2007, Finucane et al., 2006, Gil et al., 2011, Hoeinghaus et al., 2011, Hückstädt et al., 2007, Szpak et al., 2013, Szpak et al., 2014, Szpak et al., 2015, Thornton et al., 2011, Tieszen and Chapman, 1992, van Der Merwe et al., 1993). The edible portion of flora and fauna (e.g. animal muscle, plant fruit, tuber or grains, etc.) was calculated by applying suitable offsets (depending on the type of tissue and taxonomic rank) (Codron et al., 2005, Hobson and Clark, 1992, Hobson et al., 1996, Kelly, 2000, Mateo et al., 2008, Sealy et al., 1987, Sholto-Douglas et al., 1991, Vogel, 1978, Warinner and Tuross, 2010, Yoneyama and Ohtani, 1983). A correction of 1.5‰ was applied to the δ^{13}C values of modern samples to account for the Suess effect (Marino and McElroy, 1991, Schloesser et al., 2009). In Fig. 5, the tendon collagen isotope data produced in this study and the bone collagen isotope data published by Santana-Sagredo et al. (2015a) are compared against the edible portion of South American flora and fauna.

[Figure 5. Plot of δ^{15}N values versus δ^{13}C values of tendon collagen (*, this study) and bone collagen (†, Santana-Sagredo et al., 2015a) from Pica 8 individuals and of the edible portion of South American flora and fauna.]

The food groups used in the FRUITS model consist of: (1) C_4 plants (cultivated and wild), (2) marine animals (fish, sea lions, shellfish), (3) C_3 plants (fruits, vegetables, legumes), and (4) terrestrial animals (camelids, rodents and birds) consuming either C_3, C_4 or mixed C_3-C_4 plants (i.e. wild and domesticated animals living at different elevations). To account for the use of guano (seabird dung) in inland maize cultivation, a second model was run, substituting the 'C_4 plants' food group with 'maize manured with guano'. Guano was commonly imported from the coast to increase the productivity of high-altitude cultivation (Ajata López, 2013). The diet estimates generated by the FRUITS models are provided in Tables 3-4. The proportion of animal meat in reconstructed diets changes significantly between the two models. The dietary estimates of the second model (Table 4), compared to the first (Table 3), indicate a greater contribution of terrestrial animal meat and a lower contribution of marine foodstuffs. In the second model (Table 4), the proportion of marine resources is lower than that originally proposed by Santana-Sagredo et al. (2015a).

[Tables 3 and 4. Here]

The tendons of four individuals (SD-T24, SI-T32, SI-T3, SF-T4) recorded similar bulk isotope values (δ^{13}C= –7.8‰ to –8.7‰, δ^{15}N= +15.5‰ to +17.5‰, Table 2 and Fig. 5), which, according to FRUITS estimates, reflect a dietary intake based mostly on C_4 plant products (56-72% of the food intake), complemented by animal meat of terrestrial (9-34%) and/or, to a lesser extent, marine (3-15%) origin (Tables 3-4). FRUITS dietary estimates (Tables 3-4), based on tendon collagen isotope data (δ^{13}C= –18.3‰, δ^{15}N= +12.2‰, Table 2 and Fig. 5), indicate that the young woman SE-T3 consumed predominantly C_3 plant products (77-79%) and some meat of terrestrial animals (~11%) during the final months of her life, whereas the young woman SI-T74 (δ^{13}C= –10.8‰, δ^{15}N= +25.3‰, Table 2 and Fig. 5) had a significant caloric contribution from marine proteins (71-81%, Tables 3-4).

5.3. Amino acid carbon stable isotope compositions

The tendon collagen amino acid (AA) δ^{13}C values are reported in Appendix B. The δ^{13}C mass balance values, calculated based on tendon amino acid composition reported by Schofield et al. (1971), differ from the measured bulk δ^{13}C values by 0.55±0.11‰. The full dataset of hair keratin amino acid δ^{13}C values is provided in Appendix C.

The study of stable carbon isotope compositions of human proteins at the amino acid level makes it possible to track the various sources of the dietary macronutrients because of the different carbon isotope fractionations associated with the processes of assimilation and biosynthesis of different amino acids into human proteinaceous tissues. The extent of the carbon isotope fractionation between diet and bone collagen has been investigated through stable isotope analysis of tissues taken from animals raised on controlled diets (Copley et al., 2004, Hare et al., 1991, Howland et al., 2003, Jim et al., 2006, Webb et al., 2017). Essential amino acids present minimal isotope fractionation since they are assimilated directly from dietary proteins into the body tissues (Newsholme et al., 2011, Reeds, 2000). In particular, bone collagen δ^{13}C values of some essential amino acids such as leucine and phenylalanine were found to closely reflect the δ^{13}C values of the respective dietary amino acids, thus being useful for identifying the source of the protein component of the diet (Copley et al., 2004, Howland et al., 2003, Jim et al., 2006). At the base of the food chain, amino acid δ^{13}C values of C_4 plant species are enriched in the ^{13}C isotope compared with those of the C_3 plants (Fogel and Tuross, 2003), and amino acid $\delta^{13}C$ values of organic matter from marine ecosystems are enriched relative to freshwater-derived amino acids (Keil and Fogel, 2001). Non-essential amino acids can be synthesised *de novo*, in addition to direct assimilation. This means that the human body may synthesise these amino acids through metabolic pathways involving all the three macronutrients (proteins, carbohydrates, lipids) (Newsholme et al., 2011, Reeds, 2000). Among non-essential amino acids, alanine is preferentially synthesized by the body, rather than routed, even under high-protein intake (Fernandes et al., 2012, Jim et al., 2006), making this non-essential amino acid useful for identifying the non-protein portion of the diet.

A number of dietary proxies for human palaeodietary reconstruction based on bone collagen amino acid $\delta^{13}C$ values have been proposed (e.g. Choy et al., 2010, Corr et al., 2005, Honch et al., 2012, Webb et al., 2016). Arguably the greater separation of individuals belonging to four dietary groups (HMP, HFP, C4, C3, i.e. consumers of predominantly high-marine proteins, high-freshwater proteins, C4 plants, or C3 plants) was achieved using the dietary proxy $\Delta^{13}C_{\text{Val-Phe}}$ and thus by plotting $\delta^{13}C$ phenylalanine vs. $\delta^{13}C$ valine values (Honch et al., 2012). When the bone collagen $\Delta^{13}C_{\text{Val-Phe}}$ proxy (Honch et al., 2012) is applied to our dataset, the Pica individuals all fall within the range of terrestrial consumers (Fig. 6). This is difficult to reconcile with the results of the FRUITS models, as the $\Delta^{13}C_{\text{Val-Phe}}$ dietary indicator appears to underestimate the marine protein intake, especially in the case of SI-T74, who has been identified through the FRUITS models to have a diet containing a significant marine component (41% to 51%).

[Figure 6. Plot of $\Delta^{13}C_{\text{Val-Phe}}$ values for tendon collagen (this study) and bone collagen (Honch et al., 2012). HMP=high-marine protein consumer; HFP=high-freshwater protein consumer; C4=C4 plant consumers; C3=C3 plant consumers; Peru=mixed-diet group, Peru, Huari AD 500-900.]

The bi-plot $\delta^{13}C$ Phe vs. $\delta^{13}C$ Val values, proposed by Honch et al. (2012) for bone collagen, appears effective in tracking the source of the terrestrial resources in the diet of the Pica individuals, being C_3 for SE-T3 and C_4 for SD-T24, SI-T32, SF-T4 and SI-T3 (Fig. 7). However, the identification of SI-T74 as a marine resource consumer is not straightforward.

[Figure 7. Plot of $\delta^{13}C$ phenylalanine values vs. $\delta^{13}C$ valine values for tendon collagen (this study) and bone collagen (Honch et al., 2012).]

Previous studies (Choy et al., 2010, Webb et al., 2015) have shown that by using the $\delta^{13}C$ values of three amino acids the extent of separation of diverse dietary groups may be increased. In our opinion, by including a third essential amino acid, leucine, to the previous model of Honch et al. (2012) based on valine and phenylalanine $\delta^{13}C$ values, the identification of the predominant food source can be facilitated even in a mixed marine-terrestrial diet. This is because the source of carbon in the essential (bodily) amino acids phenylalanine and leucine is that of dietary proteins, even under conditions of high-lipid and low-protein diets. As a result of direct assimilation, $\delta^{13}C$ values of phenylalanine and leucine in proteinaceous tissues reflect those of the protein component of the diet with minimal isotopic fractionation (Howland et al., 2003, Newsome et al., 2011, Newsome et al., 2014). This is not true for the essential amino acid valine. Controlled feeding experiments on animals (Newsome et al., 2011, Newsome et al., 2014) have shown that carbon in valine may be sourced from the non-protein portion of the diet under conditions of low protein intake. It is suggested that gut microflora may synthesize *de novo* essential amino acids such as valine sourcing carbon from dietary carbohydrates and/or lipids (Newsome et al., 2011, Newsome et al., 2014). This implies that the δ^{13}C valine value of proteinaceous tissues may be more similar to the δ^{13}C value of the bulk diet rather than that of dietary proteins (Newsome et al., 2011), or be strongly correlated with whole diet δ^{13}C value (McMahon et al., 2010), or have a non-significant correlation with dietary amino acids (Howland et al., 2003), depending on the chosen diet. As a result of the possible processes of assimilation and/or synthesis of valine from diet to body tissues, connected to a variety of dietary types, we might expect a wide range of valine δ^{13}C values. Given these premises, we hypothesise that by using these three essential amino acids (δ^{13}C phenylalanine, δ^{13}C valine, δ^{13}C leucine), the reconstruction of palaeodiets of individuals having a mixed dietary intake will be more straightforward. The δ^{13}C values of phenylalanine and leucine may be useful in tracking and thus separating different dietary groups based on the protein component of their diet, and valine δ^{13}C values might be useful in tracking the non-protein portion of diet (carbohydrates and/or lipids), especially under the condition of protein deficiency.

[Figure 8. Proposed model for assessing the origin of the predominant dietary intake (terrestrial vs. marine), based on tendon collagen amino acid δ^{13}C values.]

As shown in Fig. 8, the δ^{13}C phenylalanine values are more negative than the leucine values for consumers of mostly marine resources (e.g. SI-T74). Conversely, the leucine δ^{13}C values are more negative than the phenylalanine values for consumers of mostly terrestrial resources (e.g. SD-T24, SI-T32, SE-T3). The range of these essential amino acid δ^{13}C values is significantly more negative for C_3 terrestrial resource consumers (e.g. SE-T3) than for the C_4 terrestrial resource consumers (e.g. SI-T32), reflecting the differential amino acid δ^{13}C values of the two plant groups (Fogel and Tuross, 2003). A similar pattern was highlighted by Honch et al. (2012) but for collagen δ^{13}C phenylalanine and valine values. However, valine presents more variable δ^{13}C values (at least in our dataset), also likely reflecting the effect of the non-protein component of the diet.

Unfortunately, the most extensive and complete dataset of human bone collagen δ^{13}C values published so far (Honch et al., 2012) does not include leucine δ^{13}C values, making it impossible to test our hypothesis further. The applicability of this Phe/Val/Leu method to various human proteinaceous tissues will need to be tested in future studies.

5.4. Dietary reconstruction of selected Pica 8 individuals

The following interpretation is based on the assumption that, even though a wide spectrum of exotic resources was available at Pica (Núñez, 1984, Uribe, 2006), it is unlikely that local individuals consumed imported foods exclusively and in a consistent manner. Consumers of exotic foodstuffs (e.g. dried fish) would be most likely to show a dietary intake made up of local and non-local products, with the proportion of the latter varying through time depending on the nature and outcome of the commerce. Based on this premise, the mobility and migratory histories of the individuals analysed are discussed, although it should be noted that these interpretations are speculative, given the possible mobility of exotic foodstuffs.

Altogether, tendon collagen amino acid δ^{13}C values and FRUITS diet estimates (Fig. 9), based on tendon collagen isotope data (δ^{13}C= –7.8‰ to –8.7‰, δ^{15}N= +15.5‰ to +17.5‰), concur in identifying that four individuals (SD-T24, SI-T32, SI-T3, SF-T4) of both sexes and different ages were consuming a terrestrial-based diet made of mostly C_4 crops (56-72% of the food intake) and animal meat (9-34%), despite being buried in different sectors (D, F, I) of the Pica 8 cemetery. According to the ecological zone in which the site is geographically located (inland, at mid-altitude, in an arid environment), it may be assumed that this was the ‘local’ diet consumed at Pica or in nearby communities. This is only partially in line with what has been discussed in previous dietary reconstructions (Santana-Sagredo et al., 2015a, Santana-Sagredo et al., 2015b) undertaken on human remains from the Chilean inland oases of Pica and Quillagua (Late Intermediate Period). Both previous studies have proposed a ‘local’ dietary intake made up of mixed terrestrial resources, being predominantly composed of maize, and marine foodstuffs. Archaeo-botanical and -faunal remains recovered from Pica-Tarapacá sites of the Late Intermediate Period include C_4 crops such as maize (*Zea mays*), rodents (*Chinchilla* sp., *Cavia porcellus*, *Lagidium viscacia*), and camelids. The marine resources had to be imported from the Pacific coast and included fish (*Trachurus symmetricus*, *Cilus gilberti*), seabirds, marine mammals, molluscs and crustaceans (*Mulinia* sp., *Oliva peruviana*, *Choromytilus chorus*, *Argopecten purpuratus*) (García and Uribe, 2012, Uribe, 2006).

Although C_3 cultigens (e.g. beans, potato, squashes, quinoa, pepper) were cultivated and C_3 fruits were gathered (*Prosopis* spp.) in Pica-Tarapacá communities of the Late Intermediate Period (García and Uribe, 2012), these plant products were only consumed by a small number of individuals buried at Pica (4 out of 35) (Santana-Sagredo et al., 2015a, Santana-Sagredo et al., 2017), at least based on the skeletons investigated so far. This is surprising as the legume tree *Prosopis* spp. is a nitrogen-fixing plant that grows well in the arid climate and saline soils of the *Pampa del Tamarugal*, being able to rely solely on groundwater (Fritz et al., 1981, Mooney et al., 1980). Based on the paucity of consumption of C_3 plants at Pica 8, it could be implied that these cultigens were less frequently cultivated in this oasis (being more easily cultivated at higher altitude in the Precordillera and Altiplano, where conditions were wetter and colder), or that they were accessed only by a selected group of people within the community, or alternatively that their cultivation was for the purpose of trade (or any of these factors to a greater or lesser extent).

[Figure 9. For each individual, from top to bottom: Calorie contribution of each food group to the diet, estimated via Bayesian stable isotope mixing model FRUITS, based on bulk δ^{13}C and δ^{15}N values of tendon collagen (this study) and bone collagen (Santana-Sagredo et al., 2015a). (Asterisk indicates FRUITS model with ‘maize fertilised with guano’ replacing ‘C_4 plants’ food group); Amino acid δ^{13}C values and calculated

$\delta^{13}C$ mass balance (MB) values for tendon collagen; Keratin $\delta^{13}C$ values of non-essential and essential amino acids, and calculated $\delta^{13}C$ mass balance (MB) values, along the hair fibre.]

Individuals SI-T3 and SI-T32 present a comparable set of tendon collagen isotope values (respectively, $\delta^{13}C$ = –8.7‰ and –8.5‰, $\delta^{15}N$= +15.5‰ and +16.1‰, $\Delta^{13}C_{Val\text{-}Phe}$= –1.7 and –0.9, $\delta^{13}C$ Phe= –16.4‰ and –17.1‰, $\delta^{13}C$ Val= –18.1‰ and –18.0‰, $\delta^{13}C$ Leu= –18.1‰ and –18.5‰, $\delta^{13}C$ mass balance= –9.1‰ and –9.0‰, Table 2 and Fig. 9) that, in accordance with FRUITS diet estimates, indicate the predominant consumption of C_4 plant products (56-69%), complemented with meat from terrestrial (14-34%) and marine (3-9%) animals (Fig. 9). The less negative $\delta^{13}C$ values of both essential and non-essential amino acids (Fig. 9), compared to those of marine (SI-T74) and C_3 (SE-T3) resource consumers, show that the adults SI-T3 and SI-T32 gleaned the majority of their proteins, carbohydrates and lipids from crops and terrestrial animal meat of C_4 origin. The keratin amino acid $\delta^{13}C$ values measured in the hair of SI-T3 and SI-T32 are also very similar (respectively, $\delta^{13}C$ Phe= –15.1‰ to –17.0‰ and –14.6‰ to –16.6‰, $\delta^{13}C$ Leu= –16.3‰ to –18.5‰ and –16.0‰ to –17.7‰, $\delta^{13}C$ Val= –13.6‰ to –16.3‰ and –13.8‰ to –15.9‰, $\delta^{13}C$ mass balance= –8.6‰ to –11.4‰ and –8.6‰ to –10.1‰, Appendix C and Fig. 9). The limited range of both essential (≤3‰) and non-essential (<5‰) amino acid $\delta^{13}C$ values confirms that all three macronutrients (proteins, carbohydrates, lipids) were predominantly retrieved from the same food source, such as C_4 resources. Given that the male SI-T3 and female SI-T32 had a consistent dietary intake typical for mid-altitude (1,300 masl) inland populations (based on local ecology), it may be speculated that these individuals were resident in the locality of Pica, or moved across similar eco-zones, throughout approximately the last year of their lives. However, FRUITS diet estimates (Tables 3-4 and Fig. 9), based on rib collagen isotope values ($\delta^{13}C$= –10.4‰, $\delta^{15}N$= +21.0‰) reported by Santana-Sagredo et al. (2015a), show that, during the previous 3 to 5 years, SI-T3 relied significantly on marine resources (22-38%), which contributed to 41-65% of his protein intake, and C_4 crops (40-48%). This dissimilarity between bone and tendon isotope values suggests that SI-T3 might have recently entered the inland community after having lived in a location with full access to marine resources (e.g. the Pacific coast and coastal valleys) or, less likely, that exotic foods were available at Pica (or preferred by this individual) only during this period of time.

The tendon collagen isotope values of the adults SD-T24 and SF-T4 (respectively, $\delta^{13}C$ = –8.2‰ and –7.8‰, $\delta^{15}N$= +16.2‰ and +17.5‰, $\Delta^{13}C_{Val\text{-}Phe}$= –0.8 and 0.0, $\delta^{13}C$ Phe= –17.2‰ and –16.8‰, $\delta^{13}C$ Val= –18.0‰ and –16.8‰, $\delta^{13}C$ Leu= –18.5‰ and –18.5‰, $\delta^{13}C$ mass balance= –8.9‰ and –8.5‰, Table 2 and Fig. 9) are broadly similar to those of the two aforementioned C_4 terrestrial resource consumers (SI-T3, SI-T32). However, the keratin amino acid $\delta^{13}C$ values (Appendix C and Fig. 9) hint at a more diverse and complex dietary intake. The range of amino acid $\delta^{13}C$ values measured in the hair of SF-T4 and SD-T24 is extensive, being as high as ~4-5‰ for phenylalanine and leucine, and ~8-9‰ for alanine, proline, aspartic acid, glutamic acid and arginine (for SD-T24). This, combined with the fact that along the hair fibres of SD-T24 and SF-T4 the $\delta^{13}C$ values of essential and non-essential amino acids change in a broadly synchronous pattern (Fig. 9), suggests that all the macronutrients (proteins, lipids, carbohydrates) were sourced from a variety of different foods that changed in proportion and quality over time. For instance, it may be inferred that several months prior to death (4-5 cm hair segment), the young male SD-T24 had a significant intake of C_3 foods, temporarily ceasing or lowering his consumption of C_4 resources, since the serine $\delta^{13}C$ value drops to –8.7‰, alanine to –15.5‰, proline to –15.9‰, arginine to –15.6‰, aspartic acid to –13.5‰, and glutamic acid to –11.6‰ (Fig. 9). FRUITS dietary estimates (Fig. 9), calculated based upon rib collagen isotope compositions published by Santana-Sagredo et al. (2015a), indicate that SD-T24 had a diet made up of predominantly C_4 crops (50-65%) complemented by terrestrial animal meat (18-38%) and C_3 plant products (10-13%) for at least several years before his death.

Although the adults SF-T4 and SD-T24 maintained a high intake of C_4 resources during approximately the last 10 months and 3 to 5 years of life, respectively, they consumed additional and diverse foods (e.g. C_3 and, to a lesser extent, marine resources) concentrated especially, but not only, during certain months. A potential explanation is that these individuals, due to their social, economic or political status had access to a vast array of resources or had control over the commercial traffic in the community. An alternative explanation is that SF-T4 (female) and SD-T24 (male) had a high degree of mobility across different eco-zones from which they derived their foods.

According to tendon collagen isotope data ($\delta^{13}C$ = –18.3‰, $\delta^{15}N$= +12.2‰, $\Delta^{13}C_{Val\text{-}Phe}$= –1.7, $\delta^{13}C$ Phe= –25.9‰, $\delta^{13}C$ Val= –27.6‰, $\delta^{13}C$ Leu= –28.3‰, Table 2 and Fig. 9), the young woman SE-T3 consumed predominantly C_3 terrestrial resources during the final months of her life. The very negative $\delta^{13}C$ values of both essential and non-essential amino acids (Fig. 9) indicate that she derived proteins, as well as carbohydrates and lipids, from C_3 foods. FRUITS dietary estimates (Fig. 9) suggest that this young adult was retrieving these three macronutrients mostly from plant products (77-79%) and also from some animal meat (~11%). Based on the comparison of tendon and rib collagen isotope data (Table 2), her dietary habits were broadly consistent at least during the last 3 to 5 years of life, although the increase in the $\delta^{15}N$ value, from +10.9‰ in bone to +12.2‰ in tendon collagen, may suggest a greater consumption of meat from terrestrial animals or, more likely, that she suffered an injury several months before death (D'Ortenzio et al., 2015, Fuller et al., 2005, Reitsema, 2013), since instances of bone fractures have been identified.

The extensive ranges of keratin $\delta^{13}C$ values (Appendix C and Fig. 9), measured in both essential (e.g. 6.5‰ for valine and 5.8‰ for phenylalanine and leucine) and non-essential (e.g. 8‰ for serine, 7.2‰ for proline, 7‰ for arginine) amino acids, suggest that the SE-T3 female retrieved dietary proteins, carbohydrates and lipids from different food sources: C_3 and C_4. However, the variations among the essential and non-essential amino acid $\delta^{13}C$ values are broadly synchronous implying that one food source was swapped for the other through time and that, if meat was consumed by SE-T3, these animals were fed on the same plant type (C_3 or C_4) as that of the plant products directly consumed by this woman. Following the changes in amino acid $\delta^{13}C$ values along her hair fibre (Fig. 9), it can be inferred that SE-T3's diet was made up of mostly C_3 resources about 10 months before death ($\delta^{13}C$ Phe= –21.2‰, $\delta^{13}C$ Leu= –23.1‰, $\delta^{13}C$ Val= –22.2‰), but the proportion of these foods declined gradually through time, being replaced by C_4 resources. However, during a specific month (4-5 cm hair segment) the source of the carbohydrates was predominantly of C_4 origin (i.e.

less negative alanine, proline and glutamic acid δ^{13}C values). Closer to the time of death, her diet was mostly composed of C_4 foods (δ^{13}C Phe= –15.8‰, δ^{13}C Leu= –17.5‰, δ^{13}C Val= –15.7‰). The onset of a new diet (i.e. rapid and drastic change) would have likely generated a sharp change in δ^{13}C values, followed by a subsequent gradual adjustment to the new diet, based on previous studies (Ayliffe et al., 2004, O'Connell and Hedges, 1999). However, this is not observed here, since the signal in SE-T3's hair is most likely the result of a gradual change in diet. Nevertheless, the fact that only 10 cm of hair has been analysed makes it possible that the change in diet happened before the time period investigated, and that the gradual change visible here (Fig. 9) is, in reality, the isotope equilibration from a C_3-based diet to the new C_4-based diet.

It remains to be ascertained whether the female SE-T3 was experiencing a gradual transition towards C_4 resources as a result of moving from a place where C_3 foods were usually produced/collected and consumed, such as the Precordillera and Altiplano, to the locality of Pica, where maize was more commonly grown and eaten (Santana-Sagredo et al., 2015a, Uribe, 2006). The fact that tendon and rib collagens present C_3 isotope signals might suggest that this young woman lived in another community, plausibly at a higher altitude, which she left several months before death. The highland origin for this individual has already been proposed by Santana-Sagredo et al. (2015a) based on the enamel δ^{18}O value (–11.3‰) measured in the 3rd molar of SE-T3. Furthermore, it cannot be excluded that this mobility pattern, spanning from the altiplano to intermediate elevations, might be linked to camelid herding practices or long-distance trade, as women were involved in these activities (Pomeroy, 2013). An alternative explanation, though less likely, is that SE-T3 was a local member of the Pica communities that had access to imported C_3 foods from the highlands, until approximately a year before death but, during the final year of life, she was only able to access C_4 resources such as maize, as a result of a change in her socio-economic status.

Given that the hair keratin recorded a transition from a C_3 to a C_4 food source, while the tendon tissues reflect a completely C_3 dietary intake likely occurring during a previous period of time, it appears that the tendon collagen is slower in turning over and adjusting to the new isotope signal than previously hypothesised (Babraj et al., 2005). A recent study by Heinemeier et al. (2013) found that the renewal of tendon tissues is quite variable within individuals, and that the turnover of the tendon core is very limited/slow. With respect to the present study, it may be speculated that the tendon of SE-T3 had poor regenerative capacity, or that only the core of the tendon has survived.

Based on tendon collagen isotope values (δ^{15}N= +25.3‰, δ^{13}C = –10.8‰, $\Delta^{13}C_{\text{Val-Phe}}$=2.0, δ^{13}C Phe= –20.6‰, δ^{13}C Val= –18.6‰, δ^{13}C Leu= –19.9‰, Table 2 and Fig. 9), it appears that towards the end of her life the young woman SI-T74 relied predominantly on marine foodstuffs, which represented 41-51% of the food she consumed and contributed to 71-81% of her protein intake (Tables 3-4). The valine δ^{13}C value of this individual is in line with those of high-marine protein consumers reported by Honch et al. (2012), but the δ^{13}C value of phenylalanine is slightly less negative, thus suggesting an additional intake of C_4 resources. Dietary estimates generated by FRUITS models confirm the concurrent consumption of some C_4 crops (36-42%), such as maize. According to tendon and rib collagen isotope compositions (Table 2), the dependence on marine resources was likely consistent over the course of the last 3 to 5 years of SI-T74's life. The slightly higher (+2.4‰) tendon δ^{15}N value (compared to that of bone) may have been induced by the consumption of manured maize and/or high trophic level marine animals, such as sea lions, or alternatively by the occurrence of a disease. Bone lesions have been identified on SI-T74's rib, which could have been generated by tuberculosis infection, although there are many other potential causes (Roberts et al., 1994; Santos and Roberts 2006).

The range of δ^{13}C values, measured along SI-T74's hair fibre, is greater than 4‰ for some essential amino acids (phenylalanine, isoleucine, lysine) and greater than 6‰ for some non-essential amino acids (alanine, glutamic acid, proline) (Appendix C and Fig. 9). This suggests that this young woman was deriving her dietary proteins from a sole source of food, such as marine resources (based on the range of essential amino acids), and that carbohydrates and lipids were also derived from another source, such as C_4 (based on the range of non-essential amino acids). The variation in amino acid δ^{13}C values along the hair fibre (Fig. 9) shows that the proportion of marine and C_4 foods changed in the diet of SI-T74 through time. Assuming a growth rate of approximately 1 cm per month (Saitoh et al., 1969) for scalp hair in the anagen phase, between about 10 to 8 months before her death this woman consumed predominantly marine resources ($\Delta^{13}C_{Val\text{-}Phe}$ =3.4 to 4.3, δ^{13}C Phe= –21.3‰ to –19.6‰, δ^{13}C Val= –17.3‰ to –16.2‰, δ^{13}C Leu= –20.1‰ to –19.1‰), while during the subsequent month (6-7 cm hair segment) she notably increased her intake of C_4 crops ($\Delta^{13}C_{Val\text{-}Phe}$ =1.7). This generated a shift of several per mill towards less negative δ^{13}C values in both essential (δ^{13}C Phe= from –21.0‰ to –17.4‰, δ^{13}C Val= from –17.3‰ to –15.7‰, δ^{13}C Leu= from –19.5‰ to –17.6‰, δ^{13}C Lys= from –13.7‰ to –11.8‰, δ^{13}C Ile= from –13.2‰ to –11.2‰) and non-essential amino acids (δ^{13}C Ala= from –14.1‰ to –8.0‰, δ^{13}C Pro= from –14.6‰ to –8.4‰, δ^{13}C Glx= from –8.7‰ to –3.8‰). Subsequently, SI-T74 decreased her intake of C_4 resources, relying mostly on marine resources ($\Delta^{13}C_{Val\text{-}Phe}$ =3.0) for a couple of months (4 to 6 cm hair segments). In the final period of her life, she then returned to a diet rich in C_4 resources ($\Delta^{13}C_{Val\text{-}Phe}$ = 1.0 to 1.8) and the δ^{13}C values of the aforementioned amino acids again became less negative (Fig. 9). Considering that the human body breaks down and recycles old proteins, as well as synthesizes new ones (O'Connell and Hedges, 1999), and that maize (the most likely consumed C_4 resource) is low in protein (although this may be slightly increased by the use of fertilisers; Keeney, 1970), it is reasonable to assume that her marine resource intake could be in reality lower than previously discussed. In other words, the marine isotope signal recorded by the keratin amino acids may derive from recycled proteins formed during a previous dietary phase characterized by high marine protein consumption, and not from a recent intake of marine resources.

Given that Pica is situated ~80 km from the sea, it is difficult to explain this consistent and significant consumption of marine resources throughout the years. Although some dried fish, fish bones and molluscs have been found at the cemetery (Núñez, 1984), it is unclear how available they would have been for regular consumption at such a distance from their source. The exchange network would need to have been developed enough such that marine resources were available routinely and in high quantities for a few selected individuals at Pica; it is therefore possible that SI-T74 had access to these foods, perhaps due to political, cultural or socio-economic reasons. An alternative explanation is that she was resident on the coast or in the coastal valleys of the Atacama Desert where marine foods and maize were both local, and only recently before death she migrated to the locality of Pica where she was eventually buried, or was travelling inland passing nearby these oases.

5. Conclusions

The individuals buried at Pica 8 present heterogeneous nutritional histories, both individually and collectively. At the time of their deaths, all six adults (SD-T24, SI-T32, SF-T4, SI-T3, SE-T3, SI-T74) were characterised by a terrestrial-C_4 diet, which is in line with what may have been the most easily accessible foodstuffs in the locality of Pica: maize and terrestrial animal meat. Among these individuals, SI-T3 and SE-T3 experienced a shift in their dietary intake several months to a year before death, possibly as a result of relocation to mid-altitude communities. The original dietary habits of SE-T3 and SI-T3 were respectively characteristic of the highlands (C_3 plants) and the coast and coastal valleys (marine resources). Only one individual (SI-T74) might have been resident on the coast or in the coastal valleys of the Atacama Desert at least over the course of the last 3 to 5 years of her life, based on a consistent intake of marine resources. However, shortly before her death SI-T74 shifted to a more terrestrial diet when she possibly migrated to, or was travelling close to, the locality of Pica.

Based on the dietary and mobility reconstruction of this subset of individuals, during the Late Intermediate Period (~1,050-500 BP), the oasis of Pica appears to have been an economically and commercially dynamic environment, which attracted people from distant regions. The individuals buried at Pica 8 may have been actively involved in the trade of exotic objects and foodstuffs, acting as traders along the caravan routes, or have belonged to the elite group who managed exchanges (Briones et al., 2005, Núñez, 1984, Pacheco and Retamal, 2017, Pomeroy, 2013). In particular, the individuals from Sector I (SI-T3, SI-T74) may have been relocated from, or linked to, communities located on the Pacific coast in order to support mutual redistribution of resources between different eco-zones (Santana-Sagredo et al., 2015a, Uribe, 2006).

To our knowledge, this is the first study that has analysed collagen amino acid δ^{13}C values in tendon samples from archaeological human remains, and this research shows that tendon may be a favourable substitute for bone in palaeodietary reconstructions, providing it is preserved in the archaeological context. Given that collagen is more abundant in tendon than in bone and its rate of turnover is faster (Babraj et al., 2005, Kjaer et al., 2005), the dietary information reconstructed based on tendon stable isotope compositions presents a higher temporal resolution and may be more fine-grained than that of bone collagen, since the original isotope signal is averaged over a shorter period of time, i.e. several months/a year instead of years/decades.

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Table 1. LC gradient program for Primesep A column (2.1x250 mm, 100 Å, 5 µm).

Time (min)	Mobile phases (%)			Flow rate (µl/min)
Conditioning run	A	B	C	
0	0	92	8	110
35	0	92	8	110
36	100	0	0	110
55	100	0	0	110
Analytical run	A	B	C	
0	100	0	0	60
45	100	0	0	60
65	60	40	0	60
75	40	25	35	60
150	0	0	100	60
180	0	0	100	60

Table 2. Bulk carbon and nitrogen isotope compositions of collagens from Pica 8 individuals.

Individual			Tendon collagen (this study)					Rib collagen (Santana-Sagredo et al., 2015a)		*Δ* Rib collagen-tendon collagen	
Burial*	Sex	Age	%N	%C	C/N	$\delta^{15}N$/‰	$\delta^{13}C$/‰	$\delta^{15}N$/‰	$\delta^{13}C$/‰	$\delta^{15}N$/‰	$\delta^{13}C$/‰
SI-T74	F	20-35 yrs	15.9 16.1	46.1 46.0	3.4 3.3	+25.3 ± 0.0	–10.8 ± 0.0	+22.9	–10.5	-2.4	0.3
SD-T24	M	20-35 yrs	16.1 16.1	45.7 45.6	3.3 3.3	+16.2 ± 0.1	–8.2 ± 0.0	+14.2	–9.6	-2.0	-1.4
SI-T32	F	35-50 yrs	15.8 15.7	45.9 46.1	3.4 3.4	+16.1 ± 0.0	–8.5 ± 0.0				
SE-T3	F	20-35 yrs	16.1 15.9	46.6 46.0	3.4 3.4	+12.2 ± 0.1	–18.3 ± 0.0	+10.9	–18.6	-1.3	-0.3
SF-T4	F	35-50 yrs	16.5 16.0	45.6 44.2	3.2 3.2	+17.5 ± 0.0	–7.8 ± 0.0				
SI-T3	M	35-50 yrs	16.0 15.5	46.6 45.1	3.4 3.4	+15.5 ± 0.0	–8.7 ± 0.1	+21.0	–10.4	5.5	-1.7

*S indicates the burial sector; T indicates the grave number.

Table 3. FRUITS model diet estimates based on the collagen isotope data from the Pica 8 individuals.

Pica	SD-T24 Tendon		SD-T24 Bone		SE-T3 Tendon		SE-T3 Bone		SF-T4 Tendon	
Food (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C3 plants	7	5	13	7	77	7	81	6	6	4
C4 plants	72	8	65	9	8	6	9	6	70	9
Terrestrial animals	11	8	18	11	11	8	7	6	9	7
Marine animals	10	4	4	3	4	3	3	2	15	5
Food fractions (%)										
Protein	27	3	29	4	30	2	27	2	28	3
Energy	73	3	71	3	70	2	73	2	72	3
Dietary proxies (Food)(%)										
$\delta^{13}C_{col}$ (C3 plants)	7	5	12	8	71	9	77	7	5	4
$\delta^{13}C_{col}$ (C4 plants)	61	10	54	11	7	5	8	5	59	11
$\delta^{13}C_{col}$ (Terr. animals)	18	11	28	15	17	12	11	8	14	9
$\delta^{13}C_{col}$ (Mar. animals)	14	6	6	4	5	4	4	3	22	7
$\delta^{15}N_{col}$ (C3 plants)	7	5	11	8	60	12	70	10	5	4
$\delta^{15}N_{col}$ (C4 plants)	39	12	34	12	4	3	5	4	37	11
$\delta^{15}N_{col}$ (Terr. animals)	30	17	45	20	27	16	19	13	23	14
$\delta^{15}N_{col}$ (Mar. animals)	24	9	10	7	9	6	6	5	35	9

Pica	SI-T3 Tendon		SI-T3 Bone		SI-T32 Tendon		SI-T74 Tendon		SI-T74 Bone	
Food (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C3 plants	9	6	16	8	8	6	12	7	15	8
C4 plants	69	9	40	9	69	9	36	7	36	8
Terrestrial animals	15	9	6	5	14	9	1	2	3	3
Marine animals	7	4	38	7	9	4	51	4	46	5
Food fractions (%)										
Protein	28	3	40	2	29	3	43	1	42	1
Energy	72	3	60	2	71	3	57	1	58	1
Dietary proxies (Food)(%)										
$\delta^{13}C_{col}$ (C3 plants)	9	6	13	6	7	6	9	5	12	6
$\delta^{13}C_{col}$ (C4 plants)	58	11	29	7	58	11	24	5	25	6
$\delta^{13}C_{col}$ (Terr. animals)	22	13	9	6	21	13	3	2	5	4
$\delta^{13}C_{col}$ (Mar. animals)	11	5	49	7	14	6	64	4	58	6
$\delta^{15}N_{col}$ (C3 plants)	8	6	9	5	7	6	6	3	8	4
$\delta^{15}N_{col}$ (C4 plants)	37	12	14	5	36	12	10	3	12	4
$\delta^{15}N_{col}$ (Terr. animals)	37	19	12	9	34	18	3	3	6	5
$\delta^{15}N_{col}$ (Mar. animals)	18	9	65	8	23	9	81	4	74	6

Energy combines the contribution of lipids and carbohydrates. Food (%) represents the calorie contribution of each food group; Fraction (%) represents the calorie contribution of each food fraction; Dietary proxy (Food)(%) represents the calorie contribution of each food group to the dietary proxies. Estimates are normalised to 100%, and uncertainty is 1-sigma (Fernandes et al., 2014, Fernandes, 2015, Fernandes et al., 2015).

Table 4. FRUITS model diet estimates based on the collagen isotope data from the Pica 8 individuals (Model with ‘maize fertilized with guano’).

Pica	SD-T24 Tendon		SD-T24 Bone		SE-T3 Tendon		SE-T3 Bone		SF-T4 Tendon	
Food (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C3 plants	6	5	10	7	79	7	84	6	5	5
Maize manured (guano)	59	7	50	6	7	5	5	4	64	7
Terrestrial animals	31	8	38	7	11	8	9	7	27	7
Marine animals	4	3	2	2	3	3	2	2	4	4
Food fractions (%)										
Protein	36	3	39	2	30	2	29	2	33	3
Energy	64	3	61	2	70	2	71	2	67	3
Dietary proxies (Food)(%)										
$\delta^{13}C_{col}$ (C3 plants)	5	5	8	6	73	9	79	8	5	4
$\delta^{13}C_{col}$ (Maize manured)	44	7	36	6	6	4	5	4	49	8
$\delta^{13}C_{col}$ (Terr. animals)	46	9	53	8	16	11	13	10	40	8
$\delta^{13}C_{col}$ (Mar. animals)	5	4	3	3	5	4	3	3	6	5
$\delta^{15}N_{col}$ (C3 plants)	4	4	6	5	63	12	70	12	4	4
$\delta^{15}N_{col}$ (Maize manured)	21	5	15	4	4	3	3	2	25	6
$\delta^{15}N_{col}$ (Terr. animals)	68	9	75	7	25	15	22	14	62	9
$\delta^{15}N_{col}$ (Mar. animals)	7	6	4	4	8	6	5	4	9	7

Pica	SI-T3 Tendon		SI-T3 Bone		SI-T32 Tendon		SI-T74 Tendon		SI-T74 Bone	
Food (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C3 plants	7	5	18	9	6	5	14	7	18	7
Maize manured (guano)	56	7	48	11	57	7	42	10	45	12
Terrestrial animals	34	7	12	7	33	7	3	2	6	5
Marine animals	3	3	22	10	4	3	41	9	31	11
Food fractions (%)										
Protein	38	3	35	3	37	3	39	2	36	3
Energy	62	2	65	3	63	2	61	2	64	3
Dietary proxies (Food)(%)										
$\delta^{13}C_{col}$ (C3 plants)	6	5	16	8	5	4	11	5	15	6
$\delta^{13}C_{col}$ (Maize manured)	41	7	38	12	42	7	31	10	35	12
$\delta^{13}C_{col}$ (Terr. animals)	49	8	16	10	48	8	4	3	8	6
$\delta^{13}C_{col}$ (Mar. animals)	4	4	30	13	5	4	54	10	42	13
$\delta^{15}N_{col}$ (C3 plants)	4	4	13	8	4	4	8	4	12	6
$\delta^{15}N_{col}$ (Maize manured)	19	5	21	10	20	5	16	8	19	10
$\delta^{15}N_{col}$ (Terr. animals)	71	8	25	13	70	8	5	5	12	9
$\delta^{15}N_{col}$ (Mar. animals)	6	5	41	16	6	5	71	10	57	15

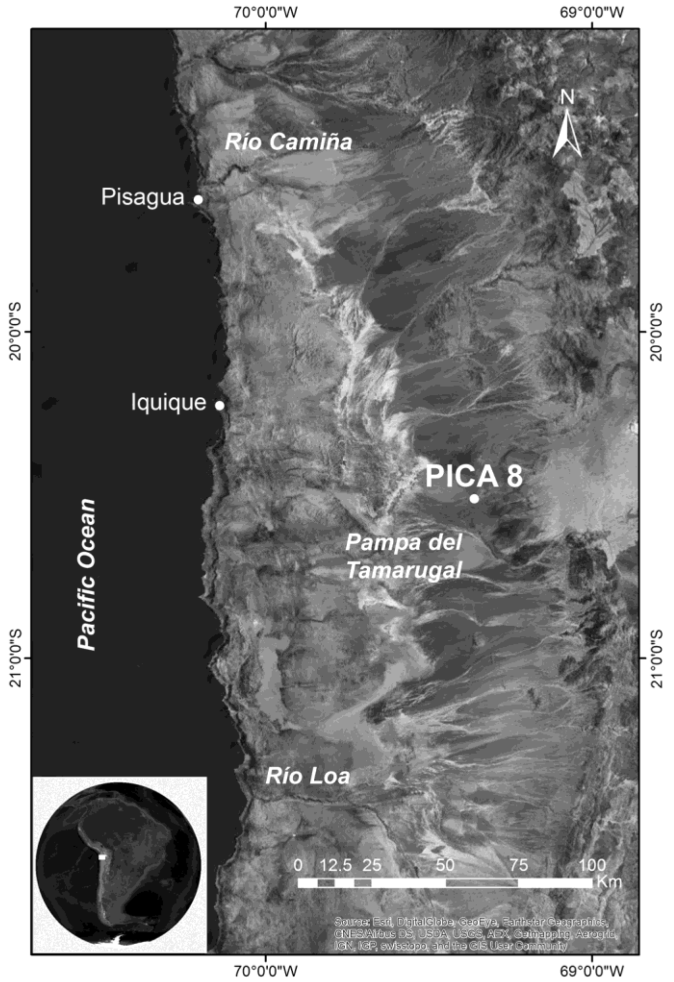


Fig 1. Map of northern Chile showing the location of the Pica 8 site.

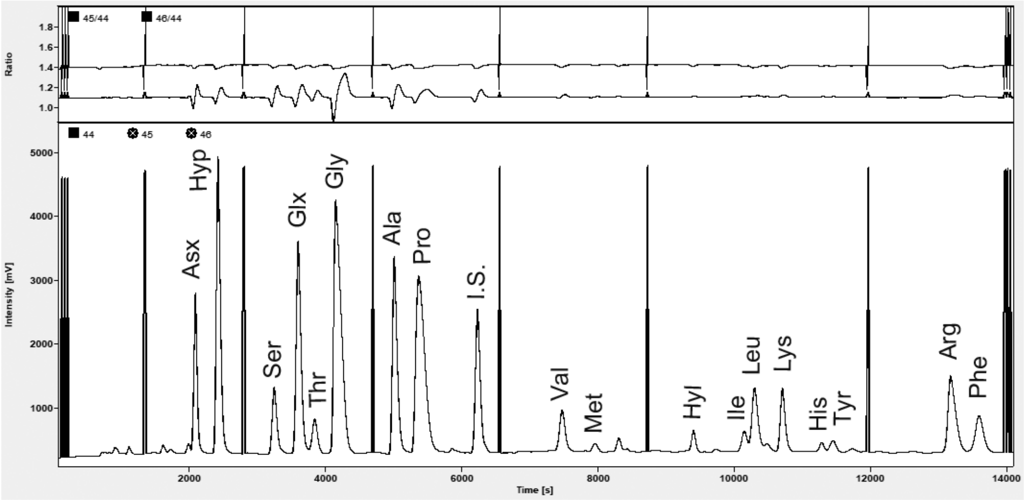


Fig 2. LC-IRMS chromatogram of tendon collagen hydrolysate (from individual SE-T3).

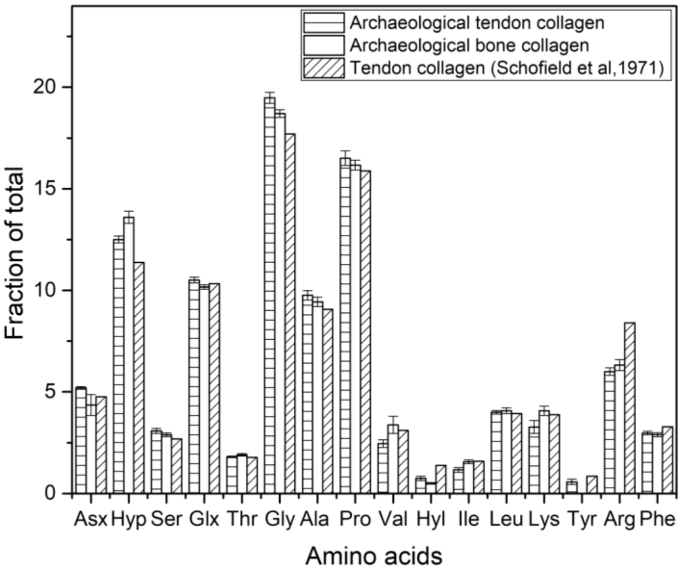


Fig 3. Fractions of the total (%) of amino acid peak areas measured in archaeological tendon collagen (this study) and bone collagen (mean±1σ), and of amino acid carbon weights (%) of human tendon collagen derived from Schofield et al. (1971).

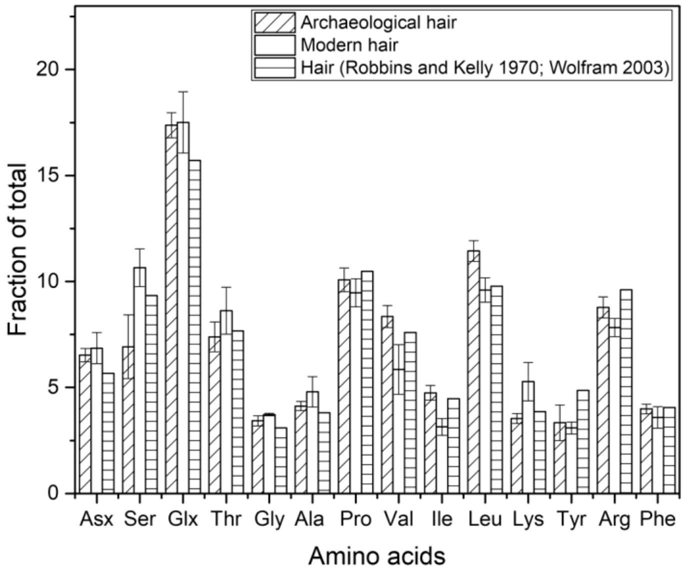


Fig 4. Fractions of the total (%) of amino acid peak areas measured in archaeological and modern hair (mean±1σ), and of amino acid carbon weights (%) measured in human hair (Robbins and Kelly, 1970; Wolfram, 2003).

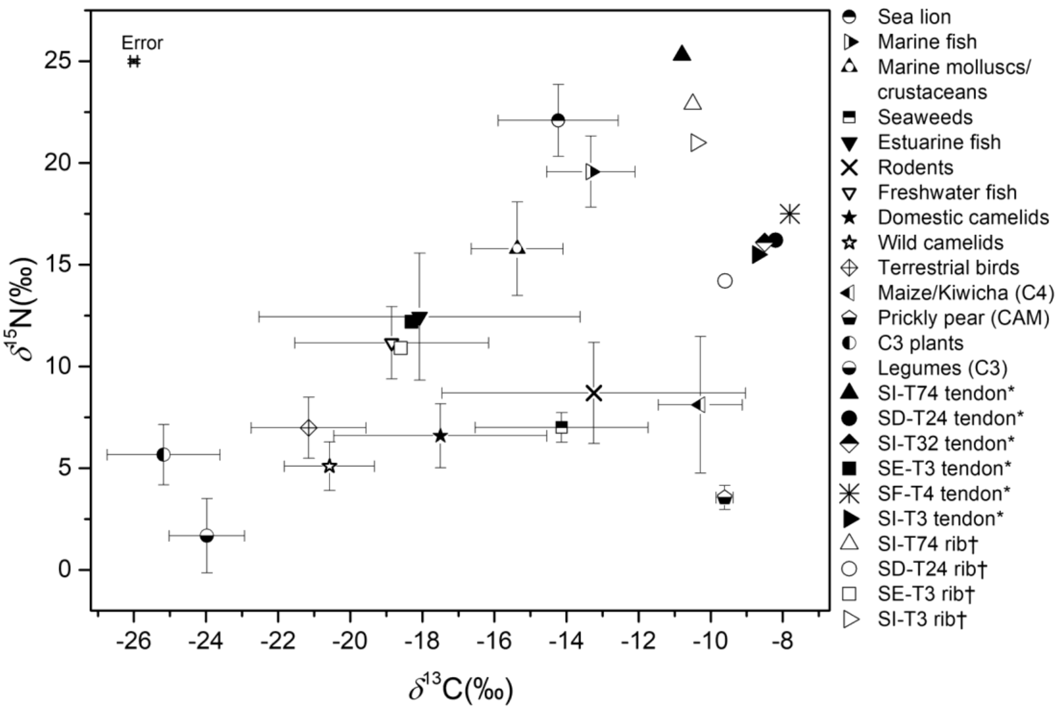


Fig 5. Plot of $\delta^{15}N$ values versus $\delta^{13}C$ values of tendon collagen (*, this study) and bone collagen (†, Santana-Sagredo et al., 2015a) from Pica 8 individuals and of the edible portion of South American flora and fauna.

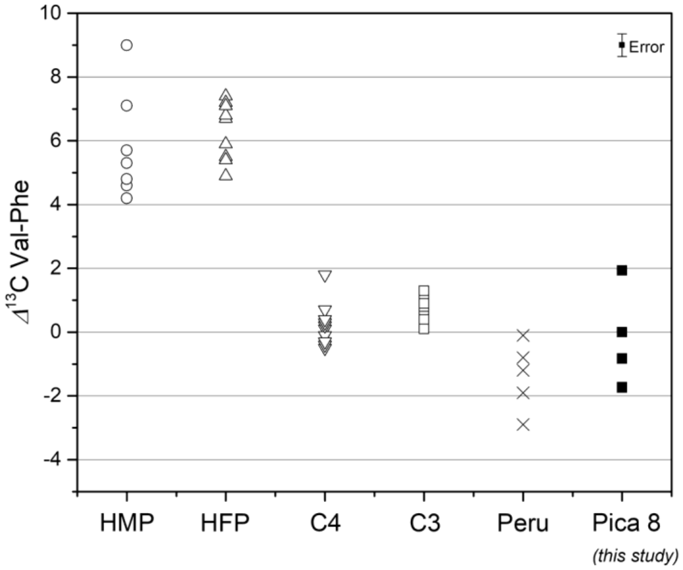


Fig 6. Plot of $\Delta^{13}C$ $_{\text{Val-Phe}}$ values for tendon collagen (this study) and bone collagen (Honch et al., 2012). HMP = high-marine protein consumer; HFP = high-freshwater protein consumer; C4=C4 plant consumers; C3=C3 plant consumers; Peru = mixed-diet group, Peru, Huari AD 500–900.

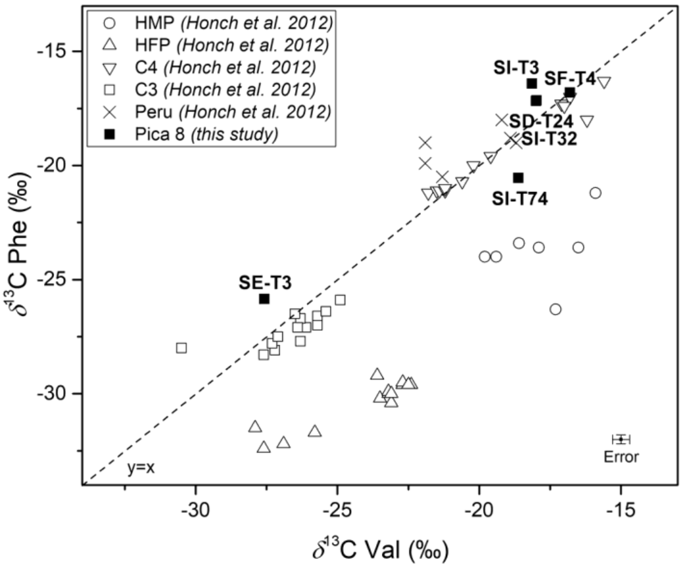


Fig 7. Plot of $\delta^{13}C$ phenylalanine values vs. $\delta^{13}C$ valine values for tendon collagen (this study) and bone collagen (Honch et al., 2012).

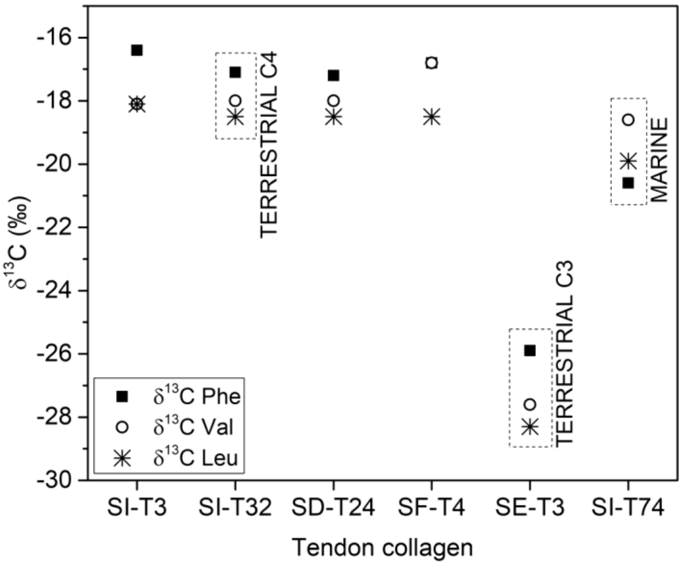
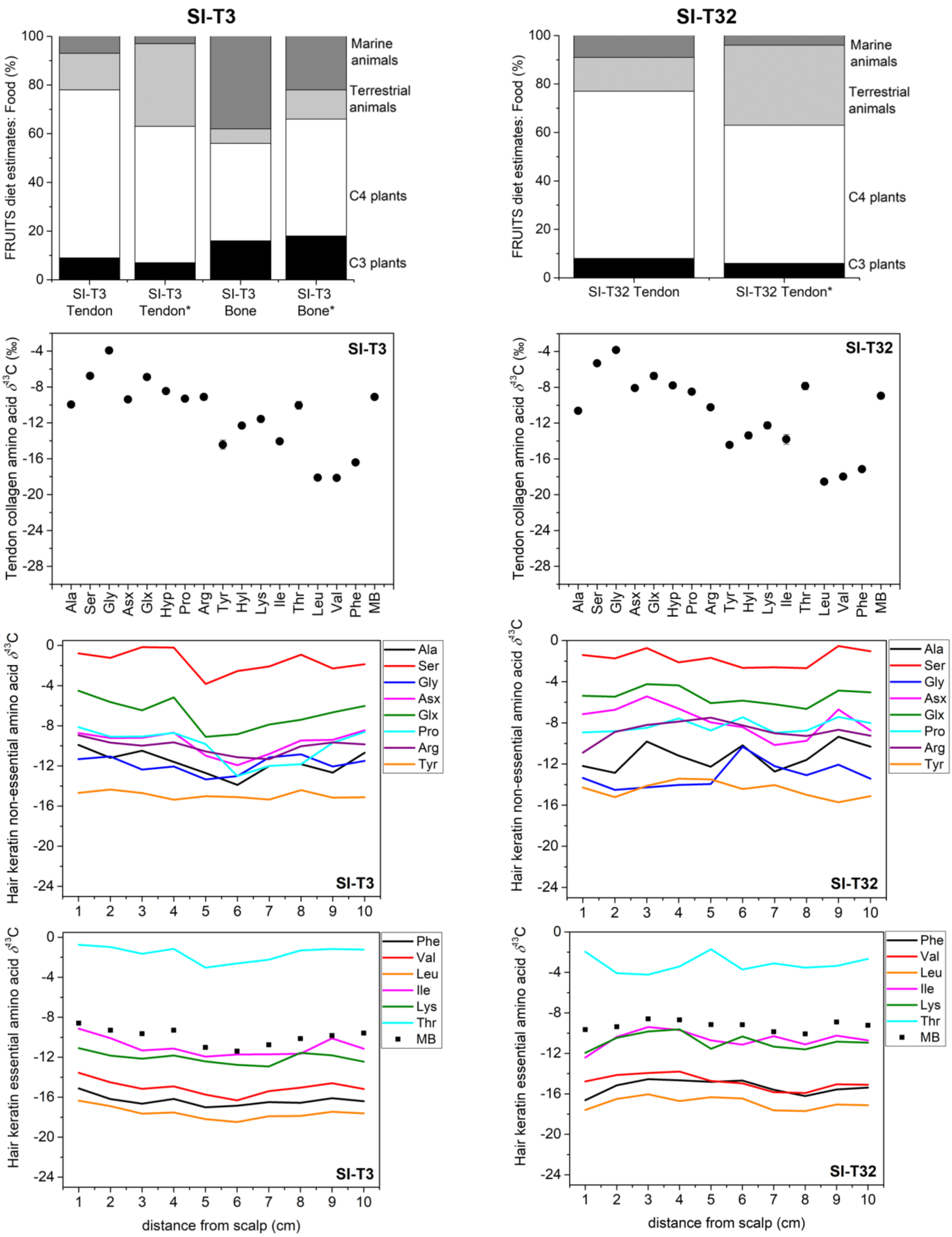
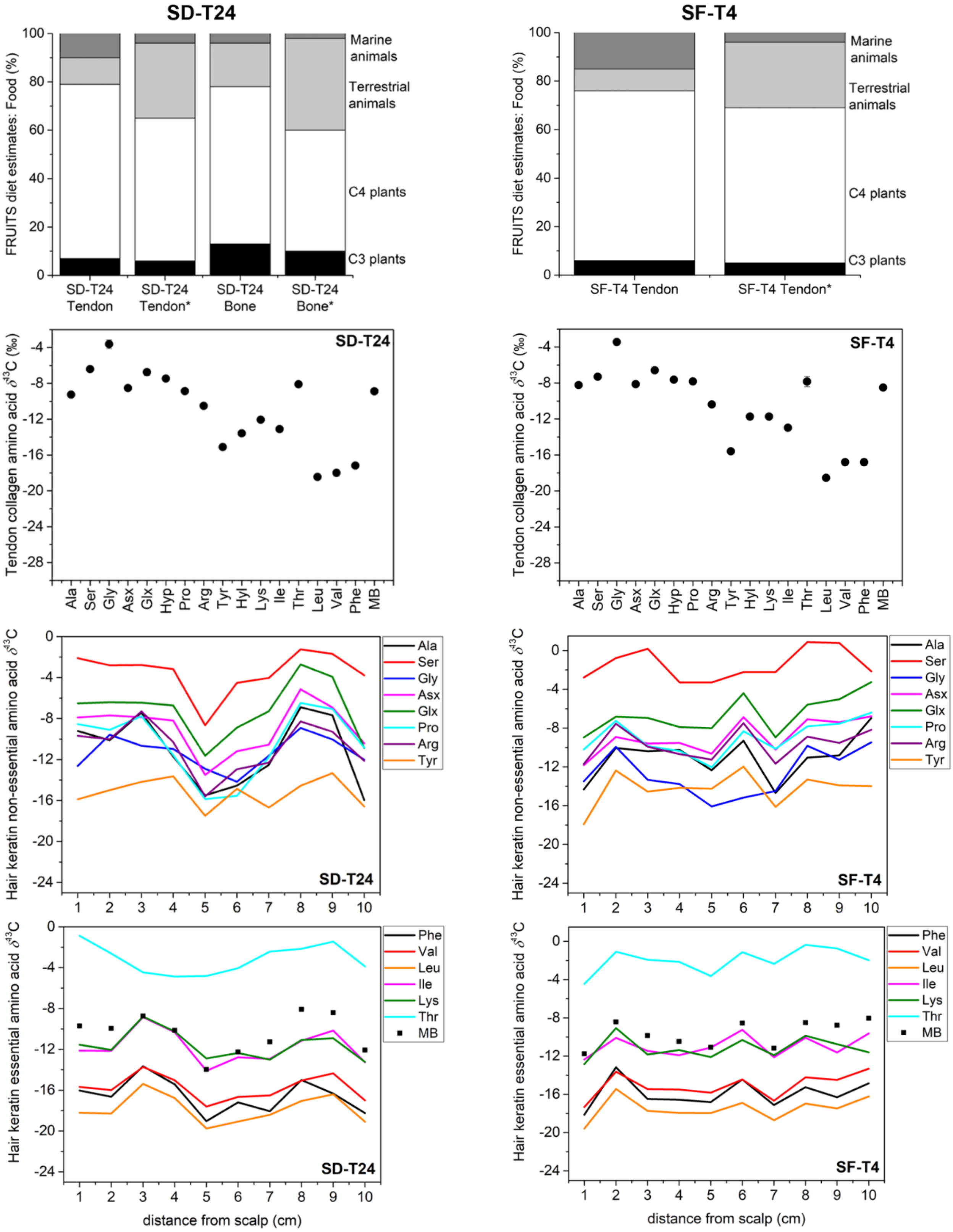


Fig 8. Proposed model for assessing the origin of the predominant dietary intake (terrestrial vs. marine), based on tendon collagen amino acid δ^{13}C values.



SI-T3
SI-T32
FRUITS diet estimates: Food (%)
Marine animals
Terrestrial animals
C4 plants
C3 plants
SI-T3 Tendon
SI-T3 Tendon*
SI-T3 Bone
SI-T3 Bone*
SI-T32 Tendon
SI-T32 Tendon*
Tendon collagen amino acid $\delta^{13}C$ (‰)
Ala
Ser
Gly
Asx
Glx
Hyp
Pro
Arg
Tyr
Hyl
Lys
Ile
Thr
Leu
Val
Phe
MB
Hair keratin non-essential amino acid $\delta^{13}C$
Hair keratin essential amino acid $\delta^{13}C$
distance from scalp (cm)



SD-T24
SF-T4
FRUITS diet estimates: Food (%)
Marine animals
Terrestrial animals
C4 plants
C3 plants
SD-T24 Tendon
SD-T24 Tendon*
SD-T24 Bone
SD-T24 Bone*
SF-T4 Tendon
SF-T4 Tendon*
Tendon collagen amino acid $\delta^{13}C$ (‰)
Ala Ser Gly Asx Glx Hyp Pro Arg Tyr Hyl Lys Ile Thr Leu Val Phe MB
Hair keratin non-essential amino acid $\delta^{13}C$
Ala Ser Gly Asx Glx Pro Arg Tyr
Hair keratin essential amino acid $\delta^{13}C$
Phe Val Leu Ile Lys Thr MB
distance from scalp (cm)

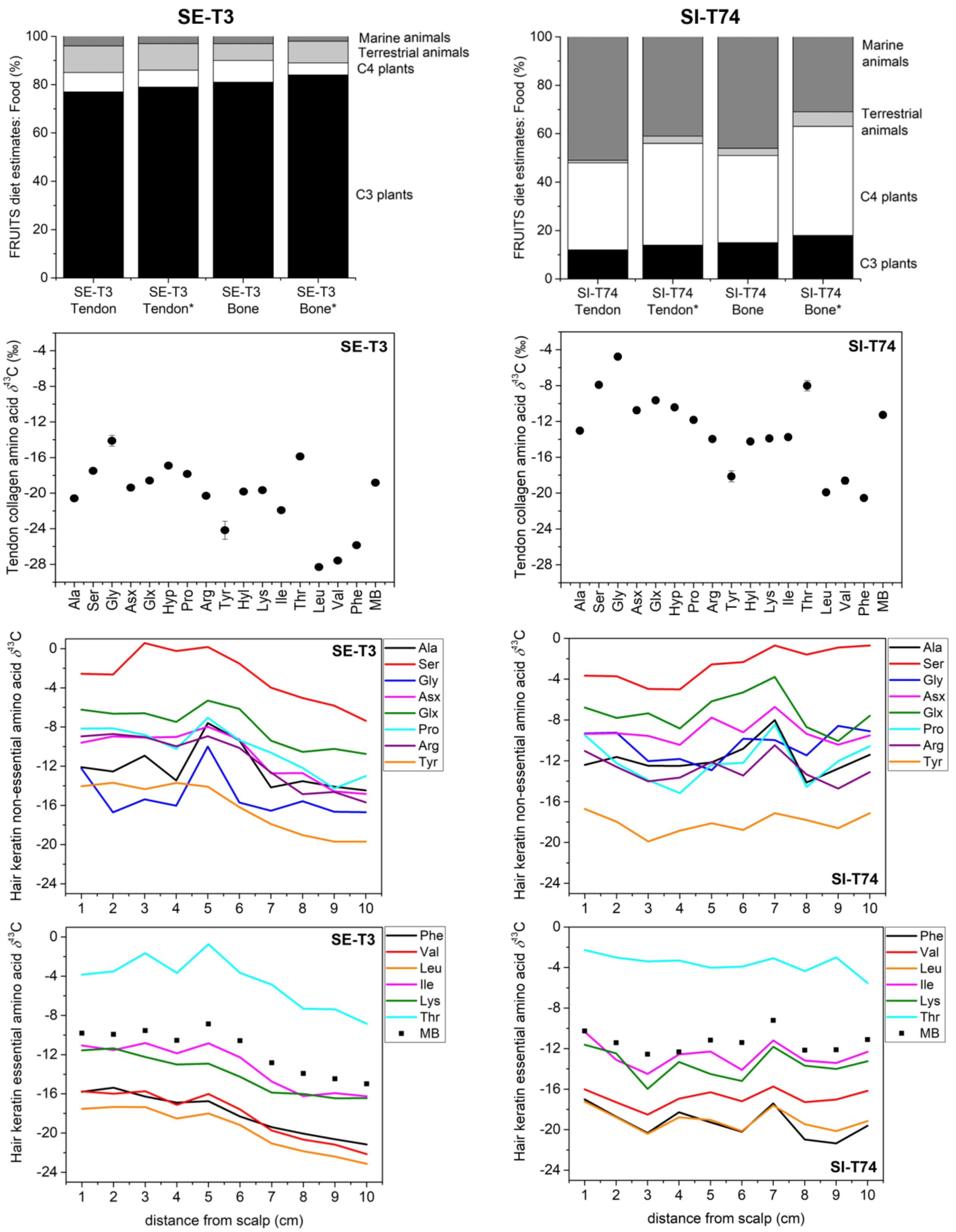


Fig 9. For each individual, from top to bottom: Calorie contribution of each food group to the diet, estimated via Bayesian stable isotope mixing model FRUITS, based on bulk $\delta^{13}C$ and $\delta^{15}N$ values of tendon collagen (this study) and bone collagen (Santana-Sagredo et al., 2015a). (Asterisk indicates FRUITS model with 'maize fertilised with guano' replacing 'C4 plants' food group); Amino acid $\delta^{13}C$ values and calculated $\delta^{13}C$ mass balance (MB) values for tendon collagen; Keratin $\delta^{13}C$ values of non-essential and essential amino acids, and calculated $\delta^{13}C$ mass balance (MB) values, along the hair fibre.

Appendix A. Supplementary data

Bayesian stable isotope mixing model: FRUITS

The Bayesian mixing model Food Reconstruction Using Isotopic Transferred Signals (FRUITS) (Fernandes et al., 2014) has been applied to the tendon collagen isotope data produced in this study and to the bone collagen isotope data published by Santana-Sagredo et al. (2015) in order to achieve an estimation of the qualitative and quantitative nutritional intake of the Pica individuals.

The dietary proxies used in the FRUITS models are the tendon and rib collagen $\delta^{15}N$ and $\delta^{13}C$ values with an associated uncertainty of 0.5‰ to account for instrumental analytical error. The food groups used include the widest array of foods available through trade from the Pacific coast, coastal valleys, mid-altitude valleys and highlands. The food groups consist of: (1) C4 plants (cultivated and wild), (2) marine animals (fish, sea lions, shellfish), (3) C3 plants (fruits, vegetables, legumes), and (4) terrestrial animals (camelids, rodents and birds) consuming either C3, C4 or mixed C3-C4 plants (i.e. wild and domesticated animals living at different elevations) (Mora et al., 2017). To account for the use of guano (seabird dung) for maize cultivation, a second model was run, substituting the 'C4 plants' food group with 'maize manured with guano' (Table A.1).

Table A.1. Isotope values and concentrations of food fractions for each food group used in the FRUITS models.

Food group	Food fraction	$\delta^{13}C$/‰	$\delta^{15}N$/‰	Concentration (%)
C3 plants	Protein	–26.7±1	+4.1±1	23±2.5
	Energy †	–24.2±1		77±2.5
C4 plants*	Protein	–12.3±1	+8.1±1	14±2.5
	Energy †	–9.8±1		86±2.5
Terrestrial animals	Protein	–17.7±1	+8.9±1	78±2.5
	Energy †	–23.7±1		22±2.5
Marine animals	Protein	–13.5±1	+19.2±1	68±2.5
	Energy †	–19.5±1		32±2.5
*Substituting food group:				
Maize fertilised with guano	Protein	–11.8±1	+22.8±1	11±2.5
	Energy †	–9.3±1		89±2.5

† Energy combines the contribution of lipids and carbohydrates

Details on calculations of isotope values and concentrations of protein, lipid and carbohydrate for each food group were reported in Mora et al. (2017) and were based on Fernandes (2015) and Newsome et al. (2004). As in Mora et al. (2017), the carbon contribution from dietary proteins has been set below 45% by applying an a priori constraint to the FRUITS model. The diet-to-tissue offset for human bone collagen has been estimated by Fernandes et al. (2012) via regression analysis performed on isotope data measured in controlled feeding experiments on animals. The statistical analysis showed that carbon in collagen is routed by about 74±4% from dietary proteins and by 26% from the energetic macronutrients (lipids and carbohydrates), while nitrogen in collagen is derived from only dietary proteins (100%) (Fernandes et al., 2012, Fernandes, 2015). The resulting diet-to-collagen offsets, which have been proposed by Fernandes et al. (2012, 2015) and used by several authors (Andrade et al., 2015, Fernandes et al., 2015), are: 4.8±0.5‰ for $\delta^{13}C$ and 5.5±0.5‰ for $\delta^{15}N$ values. Given that the contribution of the energetic macronutrients to the (bone) collagen carbon is from the three glycolytic amino acids (serine, glycine, alanine) (Fernandes et al., 2012) and that the amino acid composition of bone and tendon collagen is similar (if not identical, being both composed of type I collagen) (Eastoe, 1955), the same diet-to-collagen offsets and macronutrients' contributions will be used in the present model for the collagens extracted from bones and tendons.

It is acknowledged that it is not possible to take into account the isotope fractionation that might exist associated with the different protein metabolism and remodelling between bone and tendon. Furthermore, these FRUITS models do not account for possible alterations of the healthy metabolism induced by malnutrition or disease.

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Appendix B. Supplementary data

Amino acid $\delta^{13}C$ (‰) values (in order of LC elution), calculated $\delta^{13}C$ (‰) Mass Balance (MB) values and bulk $\delta^{13}C$ (‰) values for tendon collagen.

	Asx	Hyp	Ser	Glx	Thr	Gly	Ala	Pro	Val	Hyl	Ile	Leu	Lys	Tyr	Arg	Phe	MB	Bulk
SI-T74	–11.0	–10.5	–7.8	–9.9	–7.6	–4.9	–13.0	–12.0	–18.9	–14.1	–13.6	–19.8	–13.7	–17.7	–13.8	–20.6		
SI-T74	–10.5	–10.3	–8.0	–9.4	–8.4	–4.7	–13.1	–11.7	–18.3	–14.4	–13.9	–20.0	–14.0	–18.6	–14.2	–20.5		
mean	–10.8	–10.4	–7.9	–9.7	–8.0	–4.8	–13.0	–11.8	–18.6	–14.2	–13.7	–19.9	–13.9	–18.1	–14.0	–20.6	–11.3	–10.8
SD	0.3	0.2	0.1	0.3	0.6	0.1	0.0	0.2	0.4	0.2	0.2	0.2	0.2	0.6	0.3	0.1		
SD-T24	–8.3	–7.2	–6.5	–6.5	–7.9	–3.3	–9.1	–8.8	–18.2	–13.6	–13.3	–18.4	–12.2	–15.0	–10.5	–17.2		
SD-T24	–8.7	–7.7	–6.3	–7.0	–8.3	–4.0	–9.4	–9.0	–17.7	–13.6	–12.9	–18.5	–11.9	–15.2	–10.5	–17.2		
mean	–8.5	–7.5	–6.4	–6.7	–8.1	–3.6	–9.2	–8.9	–18.0	–13.6	–13.1	–18.5	–12.1	–15.1	–10.5	–17.2	–8.9	–8.2
SD	0.3	0.3	0.2	0.4	0.2	0.5	0.2	0.2	0.4	0.0	0.3	0.1	0.2	0.2	0.0	0.0		
SI-T32	–8.2	–8.0	–5.2	–7.0	–8.2	–3.7	–10.6	–8.5	–18.1	–13.7	–13.4	–18.3	–12.0		–10.2	–17.0		
SI-T32	–7.9	–7.6	–5.5	–6.5	–7.6	–4.0	–10.6	–8.4	–17.9	–13.1	–14.2	–18.7	–12.5	–14.4	–10.3	–17.3		
mean	–8.1	–7.8	–5.3	–6.7	–7.9	–3.8	–10.6	–8.5	–18.0	–13.4	–13.8	–18.5	–12.3	–14.4	–10.2	–17.1	–9.0	–8.5
SD	0.2	0.3	0.2	0.4	0.4	0.3	0.0	0.1	0.1	0.4	0.5	0.3	0.4		0.0	0.2		
SE-T3	–19.6	–17.1	–17.7	–18.4	–15.6	–13.7	–20.5	–17.7	–27.8	–19.6	–21.8	–28.4	–19.7	–23.5	–20.3	–25.7		
SE-T3	–19.2	–16.8	–17.3	–18.8	–16.1	–14.5	–20.7	–18.0	–27.4	–20.0	–22.1	–28.2	–19.6	–24.9	–20.3	–26.0		
mean	–19.4	–16.9	–17.5	–18.6	–15.9	–14.1	–20.6	–17.9	–27.6	–19.8	–21.9	–28.3	–19.7	–24.2	–20.3	–25.9	–18.8	–18.3
SD	0.3	0.2	0.2	0.3	0.4	0.6	0.2	0.2	0.3	0.3	0.2	0.1	0.1	1.0	0.0	0.3		
SF-T4	–8.3	–7.7	–7.3	–6.6	–8.2	–3.7	–8.0	–8.0	–17.1	–12.0	–12.8	–18.7	–11.9	–15.6	–10.5	–17.1		
SF-T4	–7.9	–7.6	–7.4	–6.6	–7.4	–3.2	–8.4	–7.7	–16.6	–11.5	–13.1	–18.4	–11.6		–10.2	–16.5		
mean	–8.1	–7.6	–7.3	–6.6	–7.8	–3.4	–8.2	–7.8	–16.8	–11.7	–13.0	–18.5	–11.7	–15.6	–10.4	–16.8	–8.5	–7.8
SD	0.3	0.1	0.1	0.0	0.6	0.3	0.3	0.2	0.3	0.3	0.2	0.3	0.2		0.2	0.4		
SI-T3	–9.6	–8.6	–6.9	–7.0	–10.4	–3.7	–9.9	–9.4	–18.3	–12.2	–14.1	–18.2	–11.8	–14.8	–9.4	–16.6		
SI-T3	–9.2	–8.3	–6.6	–6.8	–9.7	–4.2	–10.0	–9.2	–18.0	–12.4	–14.0	–18.0	–11.3	–14.1	–8.9	–16.2		
mean	–9.4	–8.5	–6.8	–6.9	–10.0	–3.9	–10.0	–9.3	–18.1	–12.3	–14.1	–18.1	–11.6	–14.4	–9.1	–16.4	–9.1	–8.7
SD	0.3	0.2	0.2	0.1	0.4	0.3	0.1	0.1	0.2	0.2	0.1	0.2	0.3	0.5	0.3	0.3		

Appendix C. Supplementary data

Table C.1. Amino acid δ^{13}C values in order of LC elution and calculated δ^{13}C Mass Balance (MB) values for hair keratin (1cm segment sequentially cut along the hair fibre starting at the root).

Individual	cm	Asx	Ser	Glx	Thr	Gly	Ala	Pro	Val	Ile	Leu	Lys	Tyr	Arg	Phe	MB
SI-T74	1	–9.2	–3.9	–6.8	–2.5	–9.8	–12.5	–9.6	–15.9	–10.4	–17.5	–11.8	–16.4	–11.3	–17.2	
SI-T74	1	–9.5	–3.5	–6.8	–2.0	–8.8	–12.3	–9.3	–16.1	–10.3	–17.0	–11.4	–17.0	–10.8	–16.8	
SI-T74	mean	–9.4	–3.7	–6.8	–2.3	–9.3	–12.4	–9.5	–16.0	–10.3	–17.2	–11.6	–16.7	–11.0	–17.0	–10.3
	SD	0.2	0.3	0.0	0.4	0.7	0.2	0.2	0.2	0.0	0.3	0.3	0.4	0.4	0.3	
SI-T74	2	–9.5	–3.7	–8.0	–3.3	–8.9	–11.8	–12.0	–17.2	–12.9	–18.8	–12.7	–18.0	–12.6	–18.9	
SI-T74	2	–9.1	–3.7	–7.6	–2.7	–9.6	–11.5	–12.4	–17.4	–13.3	–18.7	–12.3	–18.0	–12.6	–18.6	
SI-T74	mean	–9.3	–3.7	–7.8	–3.0	–9.2	–11.6	–12.2	–17.3	–13.1	–18.8	–12.5	–18.0	–12.6	–18.7	–11.4
	SD	0.3	0.1	0.3	0.4	0.6	0.2	0.3	0.2	0.3	0.1	0.3	0.0	0.0	0.2	
SI-T74	3	–9.8	–4.9	–7.3	–3.6	–12.5	–12.7	–14.2	–18.8	–14.3	–20.6	–15.7	–19.6	–14.0	–20.3	
SI-T74	3	–9.3	–5.0	–7.4	–3.1	–11.6	–12.3	–13.6	–18.2	–14.7	–20.2	–16.3	–20.2	–14.0	–20.3	
SI-T74	mean	–9.6	–5.0	–7.3	–3.4	–12.0	–12.5	–13.9	–18.5	–14.5	–20.4	–16.0	–19.9	–14.0	–20.3	–12.5
	SD	0.3	0.1	0.1	0.3	0.6	0.3	0.4	0.4	0.3	0.3	0.4	0.4	0.0	0.0	
SI-T74	4	–10.4	–5.0	–8.7	–3.7	–11.5	–12.4	–15.3	–17.0	–12.4	–19.0	–13.5	–18.8	–13.9	–18.4	
SI-T74	4	–10.5	–5.0	–9.0	–2.9	–12.1	–12.6	–15.1	–16.9	–12.8	–18.6	–13.1		–13.4	–18.1	
SI-T74	mean	–10.4	–5.0	–8.8	–3.3	–11.8	–12.5	–15.2	–16.9	–12.6	–18.8	–13.3	–18.8	–13.6	–18.3	–12.3
	SD	0.1	0.0	0.2	0.6	0.4	0.1	0.1	0.1	0.3	0.3	0.3		0.3	0.2	
SI-T74	5	–7.7	–2.4	–6.3	–3.6	–12.8	–12.0	–12.0	–16.0	–11.9	–18.7	–14.2	–18.1	–11.9	–19.0	
SI-T74	5	–7.8	–2.7	–6.1	–4.5	–13.1	–12.3	–12.7	–16.6	–12.7	–19.3	–14.8		–12.3	–19.6	
SI-T74	mean	–7.8	–2.5	–6.2	–4.0	–12.9	–12.2	–12.3	–16.3	–12.3	–19.0	–14.5	–18.1	–12.1	–19.3	–11.2
	SD	0.1	0.2	0.1	0.6	0.2	0.2	0.5	0.5	0.5	0.4	0.4		0.3	0.4	
SI-T74	6	–9.1	–2.3	–5.6	–3.8	–10.0	–10.8	–12.0	–16.9	–14.3	–20.2	–15.0	–18.5	–13.5	–20.5	
SI-T74	6	–9.3	–2.4	–5.0	–4.0	–9.7	–10.8	–12.4	–17.4	–13.9	–20.0	–15.4	–19.1	–13.4	–19.9	
SI-T74	mean	–9.2	–2.3	–5.3	–3.9	–9.8	–10.8	–12.2	–17.2	–14.1	–20.1	–15.2	–18.8	–13.4	–20.2	–11.4
	SD	0.2	0.0	0.4	0.2	0.2	0.0	0.3	0.4	0.2	0.1	0.2	0.4	0.1	0.4	
SI-T74	7	–6.6	–0.5	–3.8	–2.6	–9.6	–7.8	–8.2	–15.5	–11.0	–17.4	–11.5	–17.1	–10.3	–17.2	
SI-T74	7	–6.8	–0.9	–3.7	–3.5	–10.3	–8.2	–8.7	–16.0	–11.4	–17.8	–12.1		–10.7	–17.6	
SI-T74	mean	–6.7	–0.7	–3.8	–3.1	–10.0	–8.0	–8.4	–15.7	–11.2	–17.6	–11.8	–17.1	–10.5	–17.4	–9.2
	SD	0.2	0.3	0.1	0.6	0.5	0.3	0.3	0.4	0.3	0.2	0.4		0.3	0.3	
SI-T74	8	–9.2	–1.8	–9.0	–4.8	–11.0	–14.5	–14.8								
SI-T74	8	–9.5	–1.4	–8.4	–3.9	–11.9	–13.7	–14.3	–17.3	–13.2	–19.5	–13.7	–17.8	–13.3	–21.0	

SI-T74	mean	–9.3	–1.6	–8.7	–4.3	–11.5	–14.1	–14.6	–17.3	–13.2	–19.5	–13.7	–17.8	–13.3	–21.0	–12.2
	SD	0.2	0.3	0.4	0.6	0.7	0.6	0.3								
SI-T74	9	–10.2	–1.0	–9.9	–2.7	–9.1	–12.7	–12.1	–17.0	–13.2	–20.1	–13.8	–18.8	–14.7	–21.4	
SI-T74	9	–10.6	–0.8	–10.2	–3.3	–8.1	–12.8	–11.9	–17.0	–13.6	–20.2	–14.2	–18.4	–14.8	–21.3	
SI-T74	mean	–10.4	–0.9	–10.1	–3.0	–8.6	–12.8	–12.0	–17.0	–13.4	–20.1	–14.0	–18.6	–14.7	–21.3	–12.1
	SD	0.2	0.2	0.2	0.5	0.7	0.1	0.2	0.0	0.3	0.0	0.3	0.2	0.1	0.1	
SI-T74	10	–9.6	–0.9	–7.6	–5.5	–9.5	–11.6	–10.9	–16.3	–12.6	–19.3	–13.5	–16.8	–13.3	–19.7	
SI-T74	10	–9.4	–0.5	–7.5	–5.5	–8.7	–11.2	–10.3	–16.0	–12.0	–19.0	–13.0	–17.4	–12.9	–19.6	
SI-T74	mean	–9.5	–0.7	–7.6	–5.5	–9.1	–11.4	–10.6	–16.2	–12.3	–19.1	–13.2	–17.1	–13.1	–19.6	–11.1
	SD	0.1	0.2	0.1	0.0	0.6	0.3	0.4	0.2	0.4	0.2	0.4	0.4	0.2	0.1	
SD-T24	1	–7.7	–2.2	–6.7	–0.8	–12.3	–9.1	–8.7	–15.7	–12.4	–18.5	–11.8	–16.0	–10.0	–16.1	
SD-T24	1	–8.1	–2.0	–6.3	–0.9	–13.0	–9.4	–8.4	–15.7	–11.9	–17.9	–11.3	–15.8	–9.4	–16.0	
SD-T24	mean	–7.9	–2.1	–6.5	–0.9	–12.6	–9.2	–8.5	–15.7	–12.1	–18.2	–11.6	–15.9	–9.7	–16.0	–9.7
	SD	0.2	0.1	0.3	0.1	0.5	0.2	0.3	0.0	0.3	0.4	0.3	0.1	0.4	0.1	
SD-T24	2	–7.5	–2.6	–6.1	–2.4	–9.9	–9.8	–9.0	–15.7	–11.9	–18.0	–12.0	–15.3	–9.9	–16.4	
SD-T24	2	–7.9	–3.0	–6.7	–2.9	–9.3	–10.4	–9.2	–16.3	–12.4	–18.6	–12.2	–14.7	–10.1	–16.9	
SD-T24	mean	–7.7	–2.8	–6.4	–2.6	–9.6	–10.1	–9.1	–16.0	–12.1	–18.3	–12.1	–15.0	–10.0	–16.6	–9.9
	SD	0.3	0.3	0.4	0.4	0.4	0.4	0.2	0.4	0.4	0.4	0.2	0.4	0.1	0.4	
SD-T24	3	–7.8	–3.1	–6.7	–4.7	–10.8	–7.7	–8.1	–14.0	–9.2	–15.5	–9.1	–14.1	–7.6	–13.7	
SD-T24	3	–8.0	–2.5	–6.3	–4.3	–10.5	–7.1	–7.5	–13.4	–8.6	–15.3	–8.5	–14.2	–7.0	–13.7	
SD-T24	mean	–7.9	–2.8	–6.5	–4.5	–10.7	–7.4	–7.8	–13.7	–8.9	–15.4	–8.8	–14.2	–7.3	–13.7	–8.7
	SD	0.2	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.1	0.4	0.1	0.4	0.0	
SD-T24	4	–8.3	–3.4	–7.0	–5.2	–10.6	–11.7	–11.8	–15.3	–10.1	–17.1	–10.2	–13.4	–10.4	–15.7	
SD-T24	4	–8.1	–2.9	–6.5	–4.6	–11.4	–11.7	–11.3	–14.8	–10.6	–16.5	–10.3	–13.9	–10.1	–15.2	
SD-T24	mean	–8.2	–3.2	–6.7	–4.9	–11.0	–11.7	–11.6	–15.0	–10.3	–16.8	–10.2	–13.6	–10.3	–15.4	–10.1
	SD	0.1	0.4	0.4	0.4	0.5	0.0	0.4	0.4	0.3	0.4	0.1	0.4	0.2	0.3	
SD-T24	5	–13.4	–8.7	–12.0	–5.0	–13.2	–15.3	–16.1	–17.5	–13.9	–20.0	–13.0	–17.2	–15.5	–18.9	
SD-T24	5	–13.6	–8.6	–11.3	–4.6	–12.6	–15.7	–15.6	–17.7	–14.3	–19.5	–12.8	–17.8	–15.7	–19.2	
SD-T24	mean	–13.5	–8.7	–11.6	–4.8	–12.9	–15.5	–15.9	–17.6	–14.1	–19.8	–12.9	–17.5	–15.6	–19.0	–14.0
	SD	0.2	0.0	0.5	0.3	0.4	0.3	0.4	0.1	0.2	0.3	0.2	0.4	0.1	0.2	
SD-T24	6	–11.2	–4.4	–9.0	–4.4	–13.7	–14.8	–15.5	–16.7	–13.0	–19.2	–12.4	–14.6	–12.8	–17.3	
SD-T24	6	–11.2	–4.7	–8.8	–3.7	–14.7	–14.3	–15.6	–16.6	–12.6	–18.9	–12.3	–15.1	–13.1	–17.1	
SD-T24	mean	–11.2	–4.5	–8.9	–4.0	–14.2	–14.5	–15.5	–16.7	–12.8	–19.1	–12.4	–14.9	–12.9	–17.2	–12.3
	SD	0.0	0.2	0.1	0.5	0.7	0.3	0.1	0.1	0.2	0.2	0.0	0.3	0.2	0.2	
SD-T24	7	–10.4	–3.8	–7.6	–2.0	–11.2	–12.8	–12.1	–16.8	–13.0	–18.7	–13.3	–16.7	–12.6	–18.4	
SD-T24	7	–10.7	–4.3	–7.0	–2.9	–12.1	–12.2	–11.5	–16.2	–12.9	–18.2	–12.7		–12.0	–17.8	
SD-T24	mean	–10.5	–4.0	–7.3	–2.4	–11.6	–12.5	–11.8	–16.5	–12.9	–18.4	–13.0	–16.7	–12.3	–18.1	–11.3

	SD	0.2	0.3	0.4	0.6	0.6	0.4	0.4	0.4	0.1	0.4	0.4		0.4	0.4	
SD-T24	8	–5.1	–1.1	–2.4	–2.0	–9.1	–6.9	–6.4	–15.3	–10.8	–17.0	–11.1	–14.3	–8.0	–14.7	
SD-T24	8	–5.2	–1.4	–3.0	–2.3	–8.8	–6.9	–6.6	–14.8	–11.5	–17.1	–11.1	–14.8	–8.6	–15.3	
SD-T24	mean	–5.1	–1.2	–2.7	–2.2	–8.9	–6.9	–6.5	–15.1	–11.1	–17.1	–11.1	–14.6	–8.3	–15.0	–8.1
	SD	0.1	0.1	0.4	0.2	0.2	0.1	0.1	0.4	0.4	0.1	0.0	0.4	0.4	0.4	
SD-T24	9	–6.9		–3.7	–1.3	–9.7	–7.3	–7.3	–14.1	–10.0	–16.1	–10.8	–13.0	–9.0	–16.0	
SD-T24	9	–7.0	–1.7	–4.2	–1.6	–10.4	–8.0	–6.8	–14.6	–10.3	–16.7	–11.0	–13.7	–9.6	–16.7	
SD-T24	mean	–6.9	–1.7	–3.9	–1.4	–10.0	–7.7	–7.1	–14.3	–10.2	–16.4	–10.9	–13.3	–9.3	–16.3	–8.4
	SD	0.1		0.4	0.2	0.5	0.5	0.4	0.4	0.2	0.4	0.2	0.5	0.4	0.5	
SD-T24	10	–10.4	–4.0	–11.2	–4.0	–12.3	–16.2	–11.1	–16.9	–13.5	–19.3	–13.5	–16.5	–12.3	–18.3	
SD-T24	10	–10.4	–3.6	–10.6	–3.8	–11.7	–15.7	–10.6	–17.1	–13.0	–18.9	–13.0	–16.6	–11.9	–18.2	
SD-T24	mean	–10.4	–3.8	–10.9	–3.9	–12.0	–16.0	–10.8	–17.0	–13.3	–19.1	–13.2	–16.6	–12.1	–18.2	–12.1
	SD	0.0	0.3	0.4	0.1	0.5	0.3	0.4	0.2	0.4	0.3	0.3	0.1	0.3	0.1	
SI-T32	1	–7.0	–1.3	–5.2	–1.9	–12.9	–12.0	–8.7	–14.8	–12.4	–17.7	–12.2		–11.0	–16.5	
SI-T32	1	–7.3	–1.5	–5.5	–2.0	–13.8	–12.4	–9.2	–14.8	–12.4	–17.5	–11.6	–14.3	–10.8	–16.8	
SI-T32	mean	–7.2	–1.4	–5.4	–2.0	–13.3	–12.2	–8.9	–14.8	–12.4	–17.6	–11.9	–14.3	–10.9	–16.6	–9.6
	SD	0.2	0.1	0.2	0.0	0.6	0.3	0.4	0.0	0.0	0.1	0.4		0.2	0.2	
SI-T32	2	–6.7	–2.1	–5.5	–4.2	–14.9	–12.8	–8.8	–14.0	–10.4	–16.5	–10.7	–14.9	–9.0	–15.5	
SI-T32	2	–6.7	–1.4	–5.4	–4.0	–14.1	–12.9	–8.9	–14.3	–10.3	–16.5	–10.2	–15.5	–8.8	–14.8	
SI-T32	mean	–6.7	–1.7	–5.5	–4.1	–14.5	–12.9	–8.8	–14.1	–10.4	–16.5	–10.5	–15.2	–8.9	–15.2	–9.4
	SD	0.0	0.4	0.1	0.2	0.6	0.1	0.1	0.2	0.1	0.0	0.3	0.4	0.1	0.5	
SI-T32	3	–5.4		–4.5	–3.9	–14.4	–9.8	–8.4	–13.9	–9.8	–15.8	–9.6	–14.1	–8.5	–14.9	
SI-T32	3	–5.4	–0.7	–4.0	–4.6	–14.1	–9.8	–8.6	–14.0	–9.0	–16.2	–10.1	–14.2	–7.9	–14.2	
SI-T32	mean	–5.4	–0.7	–4.2	–4.2	–14.3	–9.8	–8.5	–13.9	–9.4	–16.0	–9.8	–14.1	–8.2	–14.6	–8.6
	SD	0.0		0.4	0.5	0.2	0.0	0.2	0.1	0.6	0.3	0.3	0.1	0.4	0.5	
SI-T32	4	–6.8	–2.1	–4.3	–3.2	–13.8	–11.3	–7.6	–13.8	–10.0	–16.7	–9.3	–13.2	–7.8	–14.5	
SI-T32	4	–6.4		–4.4	–3.6	–14.3	–11.1	–7.6	–13.8	–9.4	–16.7	–9.9	–13.7	–8.0	–14.8	
SI-T32	mean	–6.6	–2.1	–4.4	–3.4	–14.0	–11.2	–7.6	–13.8	–9.7	–16.7	–9.6	–13.4	–7.9	–14.7	–8.7
	SD	0.3		0.1	0.3	0.4	0.1	0.0	0.0	0.4	0.0	0.4	0.4	0.1	0.3	
SI-T32	5	–8.1	–1.8	–6.3	–1.4	–14.4	–12.5	–9.0	–15.0	–10.7	–16.5	–11.7	–13.5	–7.2	–14.9	
SI-T32	5	–7.8	–1.5	–5.9	–2.0	–13.5	–12.0	–8.5	–14.4	–10.7	–16.2	–11.4	–13.4	–7.7	–14.7	
SI-T32	mean	–7.9	–1.7	–6.1	–1.7	–13.9	–12.3	–8.7	–14.7	–10.7	–16.3	–11.5	–13.5	–7.5	–14.8	–9.2
	SD	0.2	0.2	0.2	0.4	0.6	0.4	0.3	0.4	0.0	0.3	0.2	0.1	0.4	0.2	
SI-T32	6	–8.2	–3.0	–6.0	–4.1	–10.2	–9.9	–7.2	–15.0	–10.9	–16.1	–10.6	–14.4	–8.5	–15.1	
SI-T32	6	–8.7	–2.3	–5.7	–3.4	–10.5	–10.5	–7.7	–14.9	–11.3	–16.8	–10.1	–14.4	–8.0	–14.3	
SI-T32	mean	–8.4	–2.7	–5.8	–3.7	–10.3	–10.2	–7.5	–15.0	–11.1	–16.5	–10.3	–14.4	–8.2	–14.7	–9.2
	SD	0.4	0.5	0.2	0.5	0.2	0.4	0.3	0.1	0.3	0.5	0.3	0.0	0.4	0.5	

SI-T32	7	–10.3	–2.8	–6.3	–3.2	–12.6	–12.6	–9.0	–16.0	–9.9	–17.8	–11.6	–14.1	–9.0	–15.4	
SI-T32	7	–10.0	–2.4	–6.1	–3.0	–11.8	–12.9	–8.9	–15.7	–10.7	–17.5	–11.1	–14.0	–9.0	–15.8	
SI-T32	mean	–10.2	–2.6	–6.2	–3.1	–12.2	–12.7	–9.0	–15.8	–10.3	–17.6	–11.3	–14.0	–9.0	–15.6	–9.9
	SD	0.2	0.3	0.2	0.2	0.6	0.2	0.1	0.3	0.5	0.2	0.4	0.1	0.0	0.3	
SI-T32	8	–9.8	–3.0	–6.3	–3.7	–13.0	–11.4	–9.0	–16.1	–11.0	–17.7	–11.9	–14.6	–9.2	–16.1	
SI-T32	8	–9.7	–2.4	–7.0	–3.4	–13.2	–11.8	–8.5	–15.7	–11.2	–17.7	–11.3	–15.4	–9.4	–16.4	
SI-T32	mean	–9.7	–2.7	–6.6	–3.5	–13.1	–11.6	–8.7	–15.9	–11.1	–17.7	–11.6	–15.0	–9.3	–16.2	–10.1
	SD	0.0	0.5	0.5	0.2	0.1	0.3	0.4	0.3	0.2	0.1	0.4	0.6	0.1	0.2	
SI-T32	9	–6.8	–0.2	–5.0	–2.9	–11.6	–9.6	–7.3	–15.0	–10.5	–17.2	–11.0	–15.7	–8.7	–15.7	
SI-T32	9	–6.6	–0.9	–4.7	–3.8	–12.5	–9.1	–7.6	–15.2	–10.0	–16.9	–10.7	–15.7	–8.6	–15.5	
SI-T32	mean	–6.7	–0.5	–4.9	–3.4	–12.1	–9.4	–7.4	–15.1	–10.2	–17.1	–10.8	–15.7	–8.7	–15.6	–8.9
	SD	0.1	0.5	0.2	0.6	0.7	0.3	0.2	0.1	0.4	0.3	0.2	0.0	0.1	0.1	
SI-T32	10	–8.7	–1.2	–5.3	–3.1	–12.9	–10.0	–7.7	–15.3	–11.0	–17.3	–10.9	–15.3	–9.0	–15.5	
SI-T32	10	–8.7	–0.9	–4.7	–2.2	–13.9	–10.6	–8.3	–14.9	–10.4	–17.0	–11.0	–14.9	–9.5	–15.3	
SI-T32	mean	–8.7	–1.0	–5.0	–2.7	–13.4	–10.3	–8.0	–15.1	–10.7	–17.1	–10.9	–15.1	–9.3	–15.4	–9.2
	SD	0.0	0.2	0.4	0.6	0.7	0.4	0.4	0.2	0.4	0.2	0.1	0.3	0.4	0.2	
SE-T3	1	–9.6	–2.7	–6.3	–4.1	–12.4	–12.3	–8.5	–15.8	–11.3	–17.8	–11.7	–14.3	–9.1	–16.0	
SE-T3	1	–9.6	–2.4	–6.2	–3.6	–12.1	–11.9	–7.8	–15.7	–10.8	–17.3	–11.4	–13.8	–8.8	–15.6	
SE-T3	mean	–9.6	–2.6	–6.2	–3.8	–12.2	–12.1	–8.2	–15.7	–11.1	–17.5	–11.6	–14.0	–8.9	–15.8	–9.8
	SD	0.0	0.2	0.1	0.3	0.3	0.2	0.4	0.1	0.4	0.3	0.2	0.3	0.2	0.3	
SE-T3	2	–8.8	–2.7	–6.9	–3.3	–16.4	–12.7	–8.0	–16.0	–11.5	–17.2	–11.1	–13.5	–8.7	–15.5	
SE-T3	2	–9.1	–2.6	–6.4	–3.7	–17.1	–12.3	–8.3	–16.0	–11.6	–17.4	–11.6	–13.9	–8.7	–15.3	
SE-T3	mean	–9.0	–2.6	–6.6	–3.5	–16.7	–12.5	–8.1	–16.0	–11.5	–17.3	–11.4	–13.7	–8.7	–15.4	–9.9
	SD	0.3	0.0	0.3	0.3	0.5	0.3	0.2	0.0	0.1	0.2	0.3	0.3	0.0	0.2	
SE-T3	3	–9.2	0.8	–6.8	–1.4	–15.1	–10.9	–8.9	–15.7	–11.0	–17.5	–12.5	–14.7	–9.2	–16.2	
SE-T3	3	–8.9	0.4	–6.4	–1.8	–15.6	–10.9	–8.7	–15.8	–10.6	–17.2	–12.0	–14.0	–8.9	–16.3	
SE-T3	mean	–9.1	0.6	–6.6	–1.6	–15.4	–10.9	–8.8	–15.7	–10.8	–17.3	–12.2	–14.3	–9.0	–16.3	–9.5
	SD	0.2	0.3	0.3	0.3	0.3	0.0	0.2	0.1	0.3	0.3	0.3	0.5	0.2	0.1	
SE-T3	4	–9.1	–0.1	–7.5	–4.0	–16.5	–13.7	–10.5	–17.3	–11.7	–18.3	–12.8	–13.5	–9.8	–16.7	
SE-T3	4	–9.0	–0.4	–7.5	–3.3	–15.5	–13.2	–9.9	–17.0	–12.1	–18.8	–13.2	–13.9	–10.1	–17.1	
SE-T3	mean	–9.0	–0.2	–7.5	–3.7	–16.0	–13.5	–10.2	–17.1	–11.9	–18.5	–13.0	–13.7	–10.0	–16.9	–10.6
	SD	0.1	0.2	0.0	0.5	0.7	0.4	0.4	0.2	0.3	0.4	0.3	0.3	0.2	0.3	
SE-T3	5	–8.1	0.1	–5.1	–0.6	–9.8	–7.8	–7.0	–16.0	–10.8	–18.0	–13.0	–13.9	–9.0	–16.6	
SE-T3	5	–7.9	0.2	–5.5	–0.9	–10.2	–7.4	–7.1	–16.0	–10.8	–18.0	–12.8	–14.3	–8.8	–16.9	
SE-T3	mean	–8.0	0.2	–5.3	–0.7	–10.0	–7.6	–7.1	–16.0	–10.8	–18.0	–12.9	–14.1	–8.9	–16.8	–8.9
	SD	0.2	0.1	0.3	0.2	0.3	0.3	0.1	0.0	0.0	0.1	0.2	0.2	0.1	0.2	
SE-T3	6		–1.2	–6.2	–3.4	–15.9	–9.2	–9.7	–17.9	–12.6	–19.5	–14.5	–16.4	–10.5	–18.6	

SE-T3	6	–9.3	–1.8	–6.1	–3.9	–15.5	–9.5	–9.0	–17.3	–12.0	–18.9	–14.0	–15.9	–9.8	–18.0	
SE-T3	mean	–9.3	–1.5	–6.1	–3.7	–15.7	–9.4	–9.3	–17.6	–12.3	–19.2	–14.3	–16.2	–10.1	–18.3	–10.6
	SD		0.4	0.0	0.3	0.3	0.2	0.5	0.4	0.4	0.4	0.4	0.4	0.5	0.4	
SE-T3	7	–12.8	–3.7	–9.5	–4.6	–16.6	–14.3	–10.5	–19.7	–14.6	–20.9	–15.7	–17.5	–12.6	–19.3	
SE-T3	7	–12.6	–4.2	–9.2	–5.1	–16.4	–14.0	–10.7	–19.8	–14.9	–21.3	–16.0	–18.3	–12.7	–19.5	
SE-T3	mean	–12.7	–4.0	–9.4	–4.9	–16.5	–14.2	–10.6	–19.7	–14.7	–21.1	–15.9	–17.9	–12.6	–19.4	–12.8
	SD	0.1	0.3	0.2	0.4	0.1	0.2	0.2	0.1	0.2	0.3	0.2	0.5	0.0	0.2	
SE-T3	8	–12.7	–4.7	–10.8	–7.0	–16.0	–13.7	–12.0	–20.8	–16.0	–21.8	–16.0	–19.0	–14.8	–20.0	
SE-T3	8	–12.7	–5.3	–10.3	–7.7	–15.1	–13.3	–12.3	–20.5	–16.5	–21.9	–16.0	–19.0	–14.9	–20.1	
SE-T3	mean	–12.7	–5.0	–10.5	–7.3	–15.6	–13.5	–12.2	–20.7	–16.2	–21.9	–16.0	–19.0	–14.8	–20.1	–13.9
	SD	0.0	0.4	0.3	0.5	0.7	0.3	0.2	0.2	0.3	0.1	0.0	0.0	0.0	0.0	
SE-T3	9	–14.7	–6.0	–9.9	–7.0	–16.5	–14.0	–14.4	–21.4	–16.1	–22.3	–16.4	–19.4	–14.7	–20.8	
SE-T3	9	–14.4	–5.6	–10.5	–7.8	–16.8	–14.2	–14.2	–21.0	–15.7	–22.4	–16.5	–20.0	–14.5	–20.5	
SE-T3	mean	–14.6	–5.8	–10.2	–7.4	–16.7	–14.1	–14.3	–21.2	–15.9	–22.4	–16.5	–19.7	–14.6	–20.6	–14.5
	SD	0.2	0.3	0.4	0.5	0.2	0.1	0.1	0.3	0.3	0.1	0.1	0.4	0.1	0.2	
SE-T3	10	–14.7	–7.3	–10.6	–8.5	–16.7	–14.6	–13.0	–22.0	–16.2	–22.9	–16.2	–19.3	–15.4	–21.0	
SE-T3	10	–15.0	–7.4	–10.9	–9.2		–14.4	–13.0	–22.3	–16.3	–23.3	–16.6	–20.1	–16.0	–21.3	
SE-T3	mean	–14.8	–7.4	–10.7	–8.8	–16.7	–14.5	–13.0	–22.2	–16.3	–23.1	–16.4	–19.7	–15.7	–21.2	–15.0
	SD	0.2	0.1	0.2	0.5		0.1	0.0	0.2	0.0	0.3	0.3	0.5	0.4	0.3	
SF-T4	1	–11.7	–2.9	–8.9	–4.3	–13.0	–14.3	–10.3	–17.4	–12.1	–19.7	–12.8	–17.8	–11.9	–18.0	
SF-T4	1	–11.9	–2.7	–9.0	–4.6	–14.0	–14.3	–10.1	–17.2	–12.6	–19.5	–12.8	–18.0	–11.5	–18.3	
SF-T4	mean	–11.8	–2.8	–9.0	–4.4	–13.5	–14.3	–10.2	–17.3	–12.3	–19.6	–12.8	–17.9	–11.7	–18.1	–11.8
	SD	0.1	0.2	0.1	0.2	0.7	0.0	0.1	0.1	0.3	0.1	0.0	0.2	0.2	0.2	
SF-T4	2	–9.0	–0.8	–6.6	–0.7	–10.3	–9.9	–7.5	–14.0	–10.3	–15.5	–9.2	–12.4	–7.3	–13.1	
SF-T4	2	–8.8	–0.7	–7.0	–1.5	–9.5	–10.2	–7.0	–13.2	–9.9	–15.4	–9.0	–12.3	–7.8	–13.3	
SF-T4	mean	–8.9	–0.8	–6.8	–1.1	–9.9	–10.0	–7.2	–13.6	–10.1	–15.4	–9.1	–12.4	–7.6	–13.2	–8.4
	SD	0.2	0.1	0.3	0.5	0.6	0.3	0.4	0.5	0.3	0.1	0.1	0.1	0.4	0.2	
SF-T4	3	–9.4		–7.0	–2.3	–13.6	–10.3	–9.7	–15.6	–11.8	–17.8	–12.0	–14.6	–10.1	–16.7	
SF-T4	3	–9.7	0.2	–6.9	–1.6	–13.1	–10.5	–10.1	–15.3	–11.1	–17.6	–11.7	–14.5	–9.7	–16.3	
SF-T4	mean	–9.6	0.2	–6.9	–1.9	–13.3	–10.4	–9.9	–15.5	–11.5	–17.7	–11.8	–14.6	–9.9	–16.5	–9.8
	SD	0.2		0.0	0.5	0.4	0.1	0.3	0.2	0.4	0.2	0.2	0.0	0.3	0.3	
SF-T4	4	–9.4	–3.4	–8.1	–2.3	–13.5	–10.0	–10.2	–15.6	–12.2	–18.2	–11.6	–14.1	–10.7	–16.6	
SF-T4	4	–9.7	–3.2	–7.7	–2.0	–14.1	–10.5	–10.5	–15.4	–11.6	–17.7	–11.1	–14.2	–10.7	–16.5	
SF-T4	mean	–9.5	–3.3	–7.9	–2.1	–13.8	–10.2	–10.4	–15.5	–11.9	–17.9	–11.4	–14.2	–10.7	–16.6	–10.5
	SD	0.2	0.1	0.3	0.2	0.4	0.4	0.2	0.2	0.4	0.3	0.3	0.1	0.0	0.1	
SF-T4	5	–10.4	–3.4	–7.8	–3.5	–16.6	–12.0	–11.9	–15.6	–10.8	–17.8	–11.9	–14.1	–11.0	–16.6	
SF-T4	5	–10.8	–3.2	–8.2	–3.7	–15.6	–12.7	–12.2	–16.0	–11.4	–18.1	–12.3	–14.4	–11.5	–17.0	

SF-T4	mean	–10.6	–3.3	–8.0	–3.6	–16.1	–12.3	–12.0	–15.8	–11.1	–18.0	–12.1	–14.3	–11.3	–16.8	–11.1
	SD	0.3	0.1	0.3	0.1	0.7	0.5	0.3	0.3	0.4	0.2	0.3	0.2	0.3	0.3	
SF-T4	6	–7.1	–1.8	–4.1	–1.6	–15.6	–9.1	–8.7	–14.4	–9.1	–17.0	–10.3	–11.7	–7.4	–14.3	
SF-T4	6	–6.7	–2.6	–4.7	–0.7	–14.7	–9.5	–8.0	–14.5	–9.4	–16.8	–10.3	–12.2	–7.6	–14.6	
SF-T4	mean	–6.9	–2.2	–4.4	–1.1	–15.2	–9.3	–8.3	–14.4	–9.3	–16.9	–10.3	–12.0	–7.5	–14.4	–8.5
	SD	0.3	0.5	0.4	0.6	0.7	0.3	0.5	0.1	0.2	0.2	0.0	0.3	0.1	0.2	
SF-T4	7	–10.4	–2.3	–9.3	–2.7	–14.2	–15.0	–10.0	–16.7	–12.0	–18.9	–12.0	–16.5	–11.6	–16.9	
SF-T4	7	–10.0	–2.2	–8.6	–2.0	–14.8	–14.3	–10.3	–16.6	–12.2	–18.5	–11.8	–15.7	–11.7	–17.4	
SF-T4	mean	–10.2	–2.2	–9.0	–2.4	–14.5	–14.7	–10.1	–16.7	–12.1	–18.7	–11.9	–16.1	–11.7	–17.1	–11.2
	SD	0.3	0.1	0.5	0.5	0.4	0.5	0.2	0.1	0.2	0.2	0.1	0.5	0.1	0.3	
SF-T4	8	–6.9		–5.7	–0.3	–10.2	–11.3	–8.1	–14.0	–10.3	–17.0	–10.2	–13.7	–9.1	–15.1	
SF-T4	8	–7.2	0.9	–5.5	–0.5	–9.5	–10.8	–7.6	–14.4	–9.8	–17.0	–9.6	–12.9	–8.7	–15.4	
SF-T4	mean	–7.1	0.9	–5.6	–0.4	–9.8	–11.0	–7.8	–14.2	–10.1	–17.0	–9.9	–13.3	–8.9	–15.3	–8.5
	SD	0.2		0.2	0.1	0.5	0.3	0.3	0.3	0.3	0.0	0.4	0.5	0.3	0.2	
SF-T4	9	–7.7	0.8	–5.2	–0.4	–11.8	–10.7	–7.8	–14.6	–11.8	–17.6	–10.7	–13.8	–9.3	–16.4	
SF-T4	9	–7.1	0.7	–4.9	–1.1	–10.8	–11.0	–7.3	–14.4	–11.4	–17.3	–10.8	–14.0	–9.7	–16.2	
SF-T4	mean	–7.4	0.8	–5.0	–0.8	–11.3	–10.8	–7.6	–14.5	–11.6	–17.5	–10.7	–13.9	–9.5	–16.3	–8.8
	SD	0.4	0.1	0.2	0.4	0.7	0.2	0.3	0.1	0.3	0.2	0.1	0.1	0.3	0.1	
SF-T4	10	–7.0	–1.9	–3.0	–1.7	–9.1	–6.7	–6.2	–13.0	–9.7	–15.9	–11.8	–14.0	–7.9	–14.6	
SF-T4	10	–6.6	–2.3	–3.5	–2.2	–9.8	–7.2	–6.6	–13.6	–9.5	–16.5	–11.4	–13.9	–8.4	–15.1	
SF-T4	mean	–6.8	–2.1	–3.3	–2.0	–9.5	–7.0	–6.4	–13.3	–9.6	–16.2	–11.6	–14.0	–8.2	–14.8	–8.0
	SD	0.3	0.3	0.4	0.3	0.5	0.3	0.2	0.4	0.1	0.4	0.2	0.1	0.3	0.4	
SI-T3	1	–8.4	–0.9	–4.6	–0.6	–11.8	–9.8	–8.2	–13.1	–8.7	–15.9	–10.9	–14.4	–8.5	–14.8	
SI-T3	1	–9.1	–0.7	–4.5	–0.9	–10.8	–10.0	–8.1	–14.0	–9.6	–16.8	–11.2	–15.0	–9.4	–15.4	
SI-T3	mean	–8.7	–0.8	–4.5	–0.8	–11.3	–9.9	–8.1	–13.6	–9.1	–16.3	–11.1	–14.7	–8.9	–15.1	–8.6
	SD	0.5	0.1	0.1	0.2	0.7	0.2	0.1	0.6	0.6	0.6	0.2	0.4	0.6	0.4	
SI-T3	2	–8.9	–1.6	–5.8	–0.9	–11.4	–11.4	–9.1	–14.3	–9.9	–16.7	–11.7	–14.2	–9.5	–15.9	
SI-T3	2	–9.5	–0.9	–5.5	–1.1	–10.7	–10.9	–9.1	–14.7	–10.3	–17.0	–12.0	–14.5	–9.9	–16.5	
SI-T3	mean	–9.2	–1.2	–5.6	–1.0	–11.1	–11.2	–9.1	–14.5	–10.1	–16.9	–11.8	–14.3	–9.7	–16.2	–9.3
	SD	0.4	0.5	0.2	0.1	0.5	0.3	0.0	0.3	0.3	0.2	0.2	0.2	0.3	0.4	
SI-T3	3	–9.0	–0.1	–6.6	–1.7	–12.9	–10.4	–9.1	–15.0	–11.2	–17.5	–11.9	–14.7	–10.0	–16.5	
SI-T3	3	–9.4	–0.2	–6.3	–1.6	–11.8	–10.5	–9.1	–15.3	–11.4	–17.8	–12.3	–14.7	–9.9	–16.8	
SI-T3	mean	–9.2	–0.2	–6.5	–1.6	–12.4	–10.5	–9.1	–15.2	–11.3	–17.6	–12.1	–14.7	–10.0	–16.7	–9.6
	SD	0.3	0.1	0.2	0.1	0.7	0.1	0.0	0.2	0.1	0.2	0.3	0.0	0.1	0.2	
SI-T3	4	–8.6	–0.4	–5.2	–0.8	–12.6	–11.8	–8.7	–14.7	–11.4	–17.4	–11.7	–15.5	–9.5	–15.9	
SI-T3	4	–8.8	–0.1	–5.2	–1.5	–11.6	–11.5	–8.7	–15.1	–10.9	–17.7	–12.0	–15.2	–9.8	–16.4	
SI-T3	mean	–8.7	–0.2	–5.2	–1.2	–12.1	–11.6	–8.7	–14.9	–11.1	–17.5	–11.8	–15.4	–9.6	–16.2	–9.3

	SD	0.2	0.2	0.0	0.5	0.7	0.2	0.0	0.3	0.4	0.2	0.2	0.2	0.3	0.3	
SI-T3	5	–10.8	–3.9	–9.3	–2.8	–13.0	–12.7	–9.7	–15.8	–11.6	–18.0	–12.1	–14.7	–10.3	–16.7	
SI-T3	5	–11.1	–3.8	–8.9	–3.2	–13.7	–12.7	–9.9	–15.7	–12.2	–18.4	–12.7	–15.3	–10.8	–17.3	
SI-T3	mean	–11.0	–3.8	–9.1	–3.0	–13.3	–12.7	–9.8	–15.7	–11.9	–18.2	–12.4	–15.0	–10.6	–17.0	–11.0
	SD	0.2	0.0	0.2	0.3	0.5	0.0	0.1	0.0	0.4	0.2	0.4	0.4	0.3	0.4	
SI-T3	6	–11.7	–2.2	–9.0	–2.6	–13.5	–13.9	–13.0	–16.3	–11.8	–18.3	–13.1	–15.1	–10.8	–16.7	
SI-T3	6	–12.1	–2.9	–8.7	–2.6	–12.5	–13.9	–13.1	–16.4	–11.6	–18.7	–12.5	–15.1	–11.4	–17.0	
SI-T3	mean	–11.9	–2.5	–8.8	–2.6	–13.0	–13.9	–13.0	–16.3	–11.7	–18.5	–12.8	–15.1	–11.1	–16.8	–11.4
	SD	0.3	0.4	0.3	0.0	0.7	0.0	0.1	0.1	0.1	0.3	0.4	0.0	0.4	0.3	
SI-T3	7	–10.8	–2.1	–7.7	–2.4	–11.3	–11.7	–11.7	–15.4	–11.4	–17.6	–12.7	–15.3	–11.3	–16.3	
SI-T3	7	–10.8	–2.1	–8.1	–2.1	–11.0	–12.3	–12.3	–15.4	–12.0	–18.2	–13.1	–15.4	–11.4	–16.7	
SI-T3	mean	–10.8	–2.1	–7.9	–2.2	–11.2	–12.0	–12.0	–15.4	–11.7	–17.9	–12.9	–15.3	–11.3	–16.5	–10.7
	SD	0.0	0.0	0.3	0.2	0.2	0.5	0.4	0.0	0.4	0.4	0.3	0.1	0.1	0.3	
SI-T3	8	–9.4	–1.1	–7.2	–1.0	–11.3	–11.6	–11.7	–15.0	–11.7	–17.7	–11.8	–14.2	–10.3	–16.6	
SI-T3	8	–9.5	–0.7	–7.6	–1.7	–10.4	–12.0	–12.0	–15.0	–11.6	–18.0	–11.3	–14.6	–9.7	–16.5	
SI-T3	mean	–9.5	–0.9	–7.4	–1.3	–10.8	–11.8	–11.8	–15.0	–11.6	–17.9	–11.6	–14.4	–10.0	–16.6	–10.1
	SD	0.1	0.3	0.3	0.5	0.6	0.3	0.2	0.0	0.1	0.2	0.3	0.3	0.4	0.1	
SI-T3	9	–9.2	–2.4	–6.9	–1.1	–11.5	–12.5	–9.5	–14.7	–10.0	–17.3	–11.5	–15.2	–9.4	–15.9	
SI-T3	9	–9.7	–2.1	–6.5	–1.3	–12.6	–12.9	–9.9	–14.5	–10.2	–17.6	–12.1	–15.2	–10.0	–16.3	
SI-T3	mean	–9.4	–2.3	–6.7	–1.2	–12.1	–12.7	–9.7	–14.6	–10.1	–17.5	–11.8	–15.2	–9.7	–16.1	–9.8
	SD	0.4	0.2	0.3	0.1	0.7	0.3	0.3	0.2	0.2	0.2	0.4	0.0	0.4	0.2	
SI-T3	10	–8.6	–2.2	–5.9	–1.3	–11.9	–10.5	–8.5	–15.1	–11.1	–17.5	–12.5	–15.0	–9.7	–16.2	
SI-T3	10	–8.4	–1.5	–6.1	–1.2	–11.1	–10.9	–8.7	–15.2	–11.2	–17.8	–12.4	–15.3	–10.0	–16.6	
SI-T3	mean	–8.5	–1.9	–6.0	–1.2	–11.5	–10.7	–8.6	–15.2	–11.1	–17.6	–12.4	–15.1	–9.8	–16.4	–9.6
	SD	0.1	0.5	0.1	0.1	0.6	0.3	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.4	

Table C.2. Summary of mean within-individual amino acid δ^{13}C (‰) values for hair keratin.

	Essential Amino Acids						Non-Essential Amino Acids								
Individual	Phe	Val	Leu	Ile	Lys	Thr	Ala	Ser	Gly	Asx	Glx	Pro	Arg	Tyr	MB*
SI-T74															
Mean	–19.3	–16.8	–19.1	–12.7	–13.6	–3.6	–11.8	–2.6	–10.4	–9.2	–7.2	–12.1	–12.8	–18.1	–11.4
SD	1.5	0.8	1.0	1.3	1.4	0.9	1.6	1.7	1.5	1.1	1.8	2.2	1.3	1.0	1.0

Range	4.3	2.8	3.2	4.2	4.4	3.2	6.1	4.3	4.3	3.7	6.3	6.8	4.2	3.2	3.3
Min	–21.3	–18.5	–20.4	–14.5	–16.0	–5.5	–14.1	–5.0	–12.9	–10.4	–10.1	–15.2	–14.7	–19.9	–12.5
Max	–17.0	–15.7	–17.2	–10.3	–11.6	–2.3	–8.0	–0.7	–8.6	–6.7	–3.8	–8.4	–10.5	–16.7	–9.2
SD-T24															
Mean	–16.6	–15.8	–17.9	–11.8	–11.6	–3.2	–11.2	–3.5	–11.4	–8.9	–7.1	–10.5	–10.8	–15.2	–10.5
SD	1.6	1.2	1.4	1.6	1.4	1.4	3.4	2.1	1.6	2.4	2.8	3.3	2.5	1.4	1.9
Range	5.3	3.9	4.4	5.2	4.4	4.0	9.1	7.5	5.3	8.4	8.9	9.4	8.3	4.2	5.9
Min	–19.0	–17.6	–19.8	–14.1	–13.2	–4.9	–16.0	–8.7	–14.2	–13.5	–11.6	–15.9	–15.6	–17.5	–14.0
Max	–13.7	–13.7	–15.4	–8.9	–8.8	–0.9	–6.9	–1.2	–8.9	–5.1	–2.7	–6.5	–7.3	–13.3	–8.1
SI-T32															
Mean	–15.3	–14.8	–16.9	–10.6	–10.8	–3.2	–11.3	–1.7	–13.1	–7.8	–5.4	–8.3	–8.8	–14.5	–9.3
SD	0.7	0.7	0.6	0.8	0.8	0.8	1.3	0.8	1.3	1.5	0.8	0.6	1.0	0.8	0.5
Range	2.0	2.1	1.7	3.0	2.3	2.5	3.5	2.2	4.2	4.8	2.4	1.6	3.4	2.3	1.5
Min	–16.6	–15.9	–17.7	–12.4	–11.9	–4.2	–12.9	–2.7	–14.5	–10.2	–6.6	–9.0	–10.9	–15.7	–10.1
Max	–14.6	–13.8	–16.0	–9.4	–9.6	–1.7	–9.4	–0.5	–10.3	–5.4	–4.2	–7.4	–7.5	–13.4	–8.6
SE-T3															
Mean	–18.1	–18.2	–19.6	–13.2	–14.0	–4.5	–12.2	–2.8	–15.2	–10.9	–7.9	–10.2	–11.3	–16.2	–11.6
SD	2.1	2.5	2.3	2.3	2.0	2.6	2.3	2.7	2.2	2.5	2.1	2.3	2.8	2.6	2.3
Range	5.8	6.5	5.8	5.5	5.1	8.1	6.9	8.0	6.7	6.8	5.4	7.2	7.0	6.0	6.1
Min	–21.2	–22.2	–23.1	–16.3	–16.5	–8.8	–14.5	–7.4	–16.7	–14.8	–10.7	–14.3	–15.7	–19.7	–15.0
Max	–15.4	–15.7	–17.3	–10.8	–11.4	–0.7	–7.6	0.6	–10.0	–8.0	–5.3	–7.1	–8.7	–13.7	–8.9
SF-T4															
Mean	–15.9	–15.1	–17.5	–11.0	–11.2	–2.0	–11.0	–1.5	–12.7	–8.9	–6.6	–9.0	–9.7	–14.3	–9.7
SD	1.5	1.3	1.2	1.1	1.1	1.3	2.3	1.6	2.4	1.8	2.0	1.8	1.6	1.7	1.4
Range	4.9	4.0	4.2	3.0	3.7	4.0	7.7	4.2	6.6	5.0	5.7	5.6	4.2	5.9	3.8
Min	–18.1	–17.3	–19.6	–12.3	–12.8	–4.4	–14.7	–3.3	–16.1	–11.8	–9.0	–12.0	–11.7	–17.9	–11.8
Max	–13.2	–13.3	–15.4	–9.3	–9.1	–0.4	–7.0	0.9	–9.5	–6.8	–3.3	–6.4	–7.5	–12.0	–8.0
SI-T3															
Mean	–16.4	–15.0	–17.6	–11.0	–12.1	–1.6	–11.7	–1.6	–11.9	–9.7	–6.8	–10.0	–10.1	–14.9	–9.9
SD	0.5	0.7	0.6	0.9	0.6	0.7	1.2	1.1	0.8	1.1	1.5	1.7	0.7	0.4	0.9
Range	1.9	2.7	2.2	2.8	1.8	2.2	4.0	3.6	2.5	3.4	4.6	4.9	2.4	1.1	2.8
Min	–17.0	–16.3	–18.5	–11.9	–12.9	–3.0	–13.9	–3.8	–13.3	–11.9	–9.1	–13.0	–11.3	–15.4	–11.4
Max	–15.1	–13.6	–16.3	–9.1	–11.1	–0.8	–9.9	–0.2	–10.8	–8.5	–4.5	–8.1	–8.9	–14.3	–8.6

*MB indicates calculated δ^{13}C mass balance values.